UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

006718

MAY 20 1988

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

Subject: Atrazine - Submission of Toxicity Studies to Fulfill the Requirements Outlined in the Registration Standard. Submitted by Ciba-Geigy Corporation December 27, 1987.
Tox. Project No.: 8-0320 Tox. Chem. No.: 63

To: Robert Taylor/Cynthia Giles
Product Manager #25
Registration Division (TS-767C)

From: Judith W. Hauswirth, Ph.D. Judith W. Hauswirth 5/19/88
Section Head, Section VI
Toxicology Branch/HED (TS-769C)

Thru: Theodore M. Farber, Ph.D., Chief 5/20/88
Toxicology Branch/HED (TS-769C)

Action Requested: Review toxicity and metabolism studies to fulfill the requirements outlined in the Atrazine registration standard.

Conclusions/Recommendations:

The DER's for each of the following studies are attached.

1. Two Generation Reproduction Study; Study No. 852063

   Parental NOEL = 50 ppm
   Parental LEL = 500 ppm based upon decreased body weights, body weight gain, and food consumption in both parental males and females throughout the study. In addition, the increase in relative testes weights seen in parental males could be treatment-related since it was seen in both generations.

   Reproductive NOEL = 10 ppm
   Reproductive LEL = 50 ppm based upon decreased body weights of pups of the second generation on postnatal day 21.

   Core Classification: Core - Minimum

2. Mouse Oncogenicity Study; Study No. 842120

   Atrazine was not oncogenic in the CD-1 mouse. The dosage levels selected for test were adequate to determine the oncogenic potential of atrazine.

1

NOEL = 300 ppm (45 mg/kg/day)
LEL = 1500 ppm (225 mg/kg/day) based upon decreases of 23.5% and 11.0% in mean body weight gain found at 91 weeks in male and female mice, respectively and an increase in the incidence of cardiac thrombi in female mice.

Core Classification: Core - Guideline

3. Chronic (52-week) Feeding Study in the Dog; Study No. 852008

NOEL = 15 ppm (0.48 mg/kg/day)
LEL = 150 ppm (4.97 mg/kg/day) based upon statistically significant decreased P-II waves in females at day 175 of study and cardiac toxicity seen in two male dogs.

At the HDT (1000 ppm), EKG alterations such as increased heart rate, decreased P-II values, atrial premature complexes, and atrial fibrillation and moderate to severe cardiac lesions (dilatation of the right atrium, atrophy and myelosis) were observed.

Core Classification: Core - Minimum

4. Dermal Absorption Studies

Study No.: ABR-83005

Core Classification: Unacceptable. Atrazine was applied in ethanol, not the field solvent, and the application site was not covered allowing material to flake off. In addition, the report was very poorly written.

Study No.: ABR-87098

Core Classification: Acceptable. Atrazine in 4L formulation is absorbed in relatively small amounts through the skin. Typical values are 2.00, 0.53 and 0.26% for 10 hour exposures to doses of 0.01, 0.1 or 1.0 mg/cm^2. Significant quantities remain on the skin after washing with soap and water (24.87, 21.10 and 10.49%). No significant differences in absorption were observed between the 4L and 80W formulations tested at 1.0 mg/cm^2 for 10 hours. The data indicate that absorption is approaching saturation at the high dose.

Study No.: ABR-83081

Core Classification: Unacceptable. The solvent tetrahydrofuran was used to dissolve atrazine for dermal application. It is not stipulated by the registrant whether tetrahydrofuran is the solvent used for field application. The dermal site of application was not covered by a material that prevents the test substance from flaking off the skin.

5. Metabolism Studies in the Rat.

The following study nurbers were submitted by the registrant to fulfill the requirement for a metabolism study in the rat:

ABR-87116	AG-520
ABR-87048	ABR-85104
ABR-87087	ABR-87115

These studies taken together are sufficient to show that in the female rat dechlorination of the triazine ring and N-dealkylation are the major metabolic pathways. Oxidation of the alkyl substituents appears to be a minor and secondary metabolic route. The total body 1/2 life is approximately one and one half days. Atrazine and/or its metabolites appear to bind to red blood cells. Other tissue accumulation does not appear to occur. The major route of excretion appears to be the urine in both male and female rats. One study indicated that approximately 75% of the administered radioactivity was excreted in the urine and approximately 20% in the feces of male and female rats given one oral dose of either 1.0 or 100.0 mg/kg atrazine or 1.0 mg/kg for seven days sub Q. However, in another study when a single oral dose of 100.0 mg of atrazine was given to female rats approximately 50% of the administered radioactivity was found in the urine and 50% in the feces.

The following must still be addressed by the registrant, concerning the metabolism of atrazine in the rat:

1. Identification of fecal metabolites in the male and female rat.
2. Identification of urinary metabolites in the male rat unless an acceptable rationale can be given that the male and female produce the same metabolites.
3. An explanation for the differences obtained in the percentage elimination of radioactiviy in the feces and urine of female rats given a single oral dose of 100 mg/kg of atrazine; the ratio was 70:25 (urine vs. feces) in one study and 50:50 in another study.

006718

Reviewed by: Judith W. Hauswirth, Ph.D., Section Head Judith W Hauswirth 4/28/88
Section VI, Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: 2-Generation Reproduction Study (83-4) TOX. CHEM. NO.: 63

MRID NO.: 404313-03

TEST MATERIAL: Atrazine

SYNONYMS: 2-Chloro-4-ethylamino-6-isopropylamino-s-triazine

STUDY NO.: 852063

SPONSOR: Ciba-Geigy Corp., Agricultural Division, Greensboro, NC 27419

TESTING FACILITY: Research Department, Pharmaceuticals Divison, Ciba-Geigy Corporation, Summit, NJ 07901

AUTHORS: J Mainiero, M Youreneff, MLA Giknis and ET Yau

REPORT ISSUED: November 17, 1987

CONCLUSION: Parental NOEL = 50 ppm
Parental LEL = 500 ppm based upon decreased body weights, body weight gain, and food consumption in both parental males and females throughout the study. In addition, the increase in relative testes weights seen in parental males could be treatment-related since it was seen in both generations.

Reproductive NOEL = 10 ppm
Reproductive LEL = 50 ppm based upon decreased body weights of pups of the second generation on postnatal day 21.

CORE CLASSIFICATION: Core - Minimum

A. MATERIALS:

1. Test compound: Atrazine, Technical. Batch FL 841802. White powder. Purity not specified but stated to be on record at Ciba-Geigy Corporation, Greensboro, NC.

2. Test animals: Species: rat; Strain: Charles River (CRCD, VAF/PLUS) from Charles River Laboratory, Ltd., Kingston, NY; Age: 37 days; Weight: males, 177-219g and females, 140-176g; animals were quarantined for one week.

B. STUDY DESIGN:

1. Animal assignment: According to the report, "One hundred twenty male and one hundred twenty female rats from the acclimation colony were randomly assigned a TEROS® temporary animal identification number and at the same time were randomly distributed into 4 treatment groups..." Permanent numbers were assigned when the animals were found acceptable for the study.

2. Experimental design: Male rats were placed on the control and test diets at 47 days of age and females at 48 days of age. They were maintained on these diets for a period of 10 weeks prior to mating. Males and females were housed together in a 1:1 ratio for mating. They were allowed a three week period for mating and were separated once evidence of mating was seen. One litter was produced in each generation. After weaning of the last litter of the first generation, thirty males and thirty females were selected for the second parental generation. The remaining male parental animals were sacrificed on days 113-114 of the study. The remaining female parental animals were sacrificed on days 133-134 of the study.

Animals selected for the second parental generation were exposed to test diets for 12 weeks prior to mating. Mating was conducted in the same manner as for the first generation. Parental males were sacrificed on day 138 of the study and parental females on days 138, 139 and 152 after weaning of their litters.

3. Test diet: Atrazine was mixed with Purina #5002 Certified Rodent Chow. The concentrations used were 0, 10, 50 and 500 ppm. Diets containing 10 to 3000 ppm atrazine were found to be stable at room temperature for at least 40 days. Periodic homogeneity analyses were performed and atrazine concentrations were found to be 93-105% of the expected values.

4. Statistics: Statistical methods can be found in Appendix 1 (Section 2.13 of the report).

5. A signed quality assurance statement was included with the study report.

C. METHODS, RESULTS, AND DISCUSSION:

1. Parental animals:

a. Observations: Animals were observed once daily for signs of toxicity and twice daily for mortality. No treatment-related clinical signs were seen in either parental generation. Alopecia and sore(s)/scab(s) were commonly seen in all groups including the controls.

At the levels tested, atrazine had no effect on mortality in either parental generation.

006718

b. Body weights: Body weights were determined weekly and at termination for males. For females, body weights were recorded weekly during the premating phase, on days 0, 7, 14 and 20 of gestation and on days 0, 4, 7, 14, and 21 of lactation. Selected body weight data can be found summarized in the following table for both parental generations.

Selected Parental Body Weight Data

Mean Body Weights (g)					
	Day				
Dose (mg/kg)	0	21	49	70	Terminal
			Males, F_0		
0	198.4	339.0	448.8	500.9	566.3
10	198.1	338.5	449.8	508.9	577.8
50	197.6	337.4	447.8	501.2	567.8
500	198.0	309.3*	396.0*	440.7*	484.8*
			Males, F_1		
0	167.8	337.6	478.4	541.1	642.3
10	160.8	329.8	471.6	528.5	626.2
50	160.6	325.2	462.6	529.6	627.4
500	146.7*	294.7*	408.9*	459.5*	540.1*
			Females, F_0		
0	158.0	220.8	261.2	281.7	
10	154.8	215.2	258.4	280.0	
50	155.2	209.8	254.5	269.9	
500	154.2	197.5*	231.6*	243.5*	
			Females, F_1		
0	141.7	212.0	262.8	287.8	
10	138.7	216.4	272.1	296.1	
50	140.1	212.4	264.5	290.4	
500	127.9*	193.7*	232.7*	251.8*	

Body Weights (Cont'd.)

006718

Females, F_0 (Gestation)			Females, F_0 (Lactation)		
	0	20	0	14	21
0	289.5	407.0	330.5	354.2	341.3
10	285.3	415.9	323.5	348.2	333.9
50	281.5	410.0	320.8	344.9	331.3
500	250.6*	376.6*	288.3*	319.1*	314.7*
Females, F_1 (Gestation)			**Females, F_1 (Lactation)**		
0	302.0	408.4	329.8	347.5	333.7
10	298.5	413.4	334.7	344.5	335.3
50	305.3	418.1	341.4	346.7	333.3
500	260.8*	370.3*	297.6*	316.9*	315.2*

* $p<0.05$

Body weights were statistically significantly lower for both males and females fed the diet containing 500 ppm atrazine (HDT) throughout the study. Body weight gains were also statistically significantly depressed at the HDT. At the mid dose (50 ppm) sporadic statistically significant decreases in body weight gain were noted. These changes are not considered to be related to treatment since they were occasional and very sporadic.

c. Food consumption: Food consumption was determined weekly for males and females during the premating period and on days 0, 7, 14, and 20 of gestation for the females.

Food consumption was statistically significantly reduced for males and females during the premating period for both parental generations and for F_1 females on days 0-7 of gestation.

d. Sacrifice and pathology: All parental animals were subjected to gross pathological examination. The testes and ovaries were weighed. The following tissues were collected for microscopic examination:

vagina	cervix	ovaries
testes	epididymides	seminal vesicles
prostate	pituitary	coagualtion gland
gross lesions		

Tissues from the control and high dose group were examined microscopically as well as <u>all</u> gross lesions.

1) Organ weights: There were no treatment-related effects on ovarian weights. Relative but not absolute testes weights were statistically significantly increased at the HDT

in parental males of both generations. The study authors attributed this change to decreased body weight gain at this dosage level.

2) Gross necropsy: No treatment-related effects were seen in either generation.

3) Histopathology: No treatment-related effects were seen in either generation.

2. Reproductive effects:

a. Pup weights: Mean pup weights per litter were recorded on lactation days 0, 4, 7, 14, and 21. Selected overall mean pup weights for each dosage group and each generationn are shown in the following table.

Mean Pup Weights (g)

F_1 Generation	Day				
Dosage Group (ppm)	0	4 (pre-culling)	7	14	21
0	6.42	9.11	14.43	31.00	49.87
10	5.99*	8.10*	12.95*	28.31*	45.09*
50	6.17	8.56	13.54	29.87	47.23
500	6.30	8.74	13.43	29.27	46.17*
F_2 Generation					
0	6.38	9.32	14.01	29.32	47.75
10	6.02*	8.75	13.39	28.26	44.55
50	6.23	9.02	13.66	28.33	43.77*
500	6.22	8.99	13.28	28.06	42.99*

* $p<0.05$

For the F_1 litter, there was a statistically significant decrease in pup body weights at the low dose (10 ppm) at all time periods recorded. Since this effect was not dose-related, this reviewer does not consider it to be due to treatment. The statisically significant decrease seen at the high dose at day 21 in body weights is also not considered, by this reviewer as well as the study author, as treatment related since it too is not dose related. However, in the F_2 generation, the statistically significant decrease in pup body weights at day 21 in the mid and high dose are considered to be treatment-related by this reviewer, since there appears to be a dose-related effect on pup body weights at this time period and in this generation.

b. External observations of pups during lactation: Pups were observed daily during lactation. No treatment-related effects

were seen.

c. Sacrifice and necropsy of pups: Pups culled on postnatal day 4 were subjected to gross necopsy as were 40 randomly selected F_2 pups on day 21. No treatment-related findings were noted.

d. Other reproductive parameters: The following reproductive paramenters were studied: number of viable litters, litter size, stillbirths, sex ratio, surival indices, male and female fertility, male and female mating index, number of pregnant females, number of implantation sites, number of viable newborns and post-implantation loss. None of these parameters was affected by treatment (see Appendix 2, Tables 6.6.3., 6.6.4., 6.8.1., 6.14.3., 6.14.4., and 6.16.1. taken from the study report).

C. CONCLUSIONS:

Atrazine at dietary levels of 10, 50, and 500 ppm had no effect on the reproductive parameters studied; however, pup weights at postnatal day 21, second generation were statistically significantly lower than those of the control group at 50 and 500 ppm. The significance of these body weight effects could have been better addressed if two litters had been produced in each generation. In the absence of this information, the reduced pup weights at this time point are considered to be treatment-related.

Body weights, body weight gain and food consumption were statistically significantly decreased for parental animals, males and females, throughout the study at the HDT. These are considered to be treatment-related effects. In addition the statistically significant increase in relative testes weights could be treatment-related, since this effect was seen in both parental generations.

Parental NOEL = 50 ppm
Parental LEL = 500 ppm based upon decreased body weight, body weight gain, and food consumption for parental animals throughout the study. In addition, the increase in relative testes weights could be treatment-related, since this effect was seen in parental males of both generations.

Reproductive NOEL = 10 ppm
Reporductive LEL = 50 ppm based upon decreased body weight of pups on postnatal day 21 in the second generation.

D. CORE CLASSIFICATION: Core-Minimum

Appendix 1

2.13. Statistical Procedures: Statistical analyses were performed as indicated below or as indicated in the individual reports from Research Statistics Services:

Statistical Analyses and References

006718

1)	Parameters:	Parental Body Weight, Body Weight Gain, Feed Consumption, Absolute and Relative Organ Weight.
	Statistical Methods:	One-way Analysis of Variance (ANOVA, Snedecor and Cochran, 1968), Bartlett's Test for Homogeneity of Variance (Snedecor and Cochran, 1968) and Dunnett's Method of Multiple Comparisons between control and treatment groups (Dunnett, 1955, 1964). Statistically significant differences between treatment groups (e.g. low dose vs. high-dose group) are not discussed. The calculations derived from the ANOVA are employed in the subsequent Bartlett's and Dunnett's Tests. The primary focus of these analyses is on the results of the Dunnett's comparisons between the control and each of the treated groups.
2)	Parameters:	Precoital Interval, maternal gestation duration, implantations, stillbirths, post-implantation loss, % post-implantation loss, survival indices, fertility indices and pup sex ratios (% males)
	Statistical Methods:	Refer to the Research Statistics Services reports located in Appendices 7.5.3. and 7.6.6.
3)	Parameter:	F_1 and F_2 Generation pup body weights
	Statistical Methods:	Healy Analysis (Healy, 1972)

In general the values presented in the tables (see Section 6) are with outliers included. If statistically significant effects result from reanalysis with the outliers excluded, then these values are presented in the tables.

2.14. Numerical Significance: Floating point arithmetic and rounding were used in all TEROS® Computer System computations as illustrated in the following example.

Parameter	Data	Raw Data	TEROS® Reports: Individual Data	Derived Means	Statistical Reports
Body Weight (grams)	230	230	230.00	230.46	230.4643
Uterine Weights (grams)	12.22	12.2	12.2	NA	NA
Feeder Weight/ Consumption (grams)	400	400	20.4	20.45	20.4545

Appendix 2

TABLE 6.6.3.

Summary of the F_0 Generation Reproductive Parameters

(Mean ± Standard Deviation)[a]

Parameter	Dietary Level (ppm)			
	Control (0)	10	50	500
Number of pregnant females	29	28	26	26
Number of viable litters[d]	28[d]	28	26	25[d]
Number of implantation sites	14.28 ± 3.53	15.68 ± 2.09	15.38 ± 3.11	14.88 ± 4.03
Number of stillbirths	0.26 ± 0.53[c]	0.25 ± 0.44	0.23 ± 0.51	0.35 ± 0.69
Number of viable newborns	13.19 ± 3.58[c]	15.04 ± 2.05	14.96 ± 2.13	13.65 ± 4.55
Post-implantation loss[b]	1.59 ± 1.58 [c]	1.04 ± 1.26	0.96 ± 1.28	1.42 ± 1.92
% Post-implantation loss[b]	13.08 ± 20.00	6.41 ± 7.66	5.35 ± 6.87	11.47 ± 20.70

[a]Although means and standard deviations are reported, the actual statistical analysis is based on non-parametric methods. See Appendices 7.6.3. and 7.10.4.2.

[b]When the number of viable pups exceeded the number of implants, the post-implantation loss was calculated as zero to ensure meaningful biological interpretation of the statistical analysis.

[c]Number of dams examined = 27

[d]Control female No. 1722 did not deliver a litter but was found to have 3 uterine implantation sites at necropsy.

500 ppm female No. 4720 - delivered only 2 stillborn pups.

TABLE 6.6.4.

Summary of the F_0 Generation Reproductive and Fertility Indices

Proportions (Percents)

Parameter	Dietary Level (ppm)			
	Control (0)	10	50	500
Female fertility	29/30 (96.7)	28/30 (93.3)	26/30 (86.7)	26/30 (86.7)
Female mating index	30/30 (100)	30/30 (100)	30/30 (100)	30/30 (100)
Gestation index	28/29 (96.6)	28/28 (100)	26/26 (100)	25/26 (96.2)
Male fertility	29/30 (96.7)	28/29 (96.6)	26/30 (86.7)	26/30 (86.7)
Male mating index	30/30 (100)	29/30 (96.7)	30/30 (100)	30/30 (100)

See Appendix 7.6.3.

Atrazine Technical
MIN 852063
A Two Generation Study in Rats

TABLE 6.8.1.

Summary of F_1 Litter Size (Day 0 Lactation) Survival Indices and Sex Ratios[a]

Parameter	Dietary Levels (ppm) Control (0)	10	50	500
Number of viable litters[a]	28 (26)	28	26	25
Mean litter size (day 0 lactation)	13.69	15.04	14.96	14.20
Mean no. stillbirths	0.22	0.25	0.19	0.27
Number of viable males (day 0)	166	218	206	171
Number of viable females (day 0)	190	203	183	184
Sex ratio day 0 Lactation: (% males)	46.6	51.8	53.0	48.2
Survival Indices - Sexes Pooled:				
Mean % pups surviving day 0-4 (pre-cull)	96.6	96.9	97.7	95.7
Mean % pups surviving day 4-21 (post-cull)	97.6	91.1	96.2	98.0

(continued)

[a] 0 ppm — 28 viable litters were born, however, Nos. 1704 and 1711 were excluded due to questionable day 0 lactation date.
No. 1722 - No viable litter
No. 1723 - Not pregnant

10 ppm — Nos. 2719 and 2721 not pregnant.

50 ppm — Nos. 3702, 3705, 3715, and 3717 not pregnant.

500 ppm — Nos. 4709, 4712, 4718 and 4721 not pregnant.
No. 4720 - delivered 2 stillborn pups.

See Appendices 7.6.3., 7.10.2., and 7.10.4.2.

TABLE 6.14.3.

Summary of F_1 Generation Reproductive Parameters
(Mean ± Standard Deviation)[a]

Parameter	Treatments (ppm)			
	Control (0)	10	50	500
Number of Pregnant Females:	24	18	28	26
Number of viable litters:[c]	23	18	28	25
Number of implantation sites	12.12 ± 5.18	13.72 ± 3.77	12.82 ± 4.10	13.68 ± 3.35
Number of stillbirths	0.50 ± 0.83	0.17 ± 0.51	0.07 ± 0.26	0.31 ± 0.74
Number of viable newborns[c]	11.00 ± 4.97	13.33 ± 2.81	13.43 ± 3.27	12.35 ± 4.08
Post-implantation loss[b]	1.29 ± 1.46	1.44 ± 1.79	0.75 ± 1.08	1.56 ± 2.18
% Post-implantation loss[b]	13.97 ± 21.67	9.71 ± 11.86	4.97 ± 7.18	12.22 ± 20.38

[a]Although means and standard deviations are reported, the actual statistical analysis is based on non-parametric methods. See Appendices 7.6.6. and 7.10.9.2.

[b]When the number of viable pups exceeded the number of implants, the post-implantation loss was calculated as zero to ensure meaningful biological interpretation of the statistical analysis.

[c]Control: Female No. 1755 did not deliver a litter but was found to have one uterine implantation site at necropsy.

500 ppm: Female No. 4738 did not deliver but was found to have 10 uterine implantation sites at necropsy.

TABLE 6.14.4.

Summary of F_1 Generation Reproductive and Fertility Indices

Proportions (percents)

Parameter	Treatments (ppm)			
	Control (0)	10 ppm	50 ppm	500 ppm
Female fertility	24/28 = (85.7)	18/26 = (69.2)	28/30 = (93.3)	26/29 = (89.7)
Female mating index	28/30 = (93.3)	26/30 = (86.7)	30/30 = (100.0)	29/30 = (96.7)
Gestation index	23/24 = (95.8)	18/18 = (100.0)	28/28 = (100.0)	25/26 = (96.2)
Male fertility	24/28 = (85.7)	18/26 = (69.2)	28/30 = (93.3)	26/29 = (89.7)
Male mating index	28/30 = (93.3)	26/30 = (86.7)	30/30 = (100.0)	29/30 = (96.7)

See Appendix 7.6.6.

006718

Atrazine Technical
MIN 852063
A Two Generation Study in Rats

Table.6.16.1.

Summary of F_2 Litter Size (Day 0 Lactation), Survival Indices and Sex Ratios

Parameter	Dietary Level (ppm)			
	Control (0)	10	50	500
Number of Viable Litters[a]	23	18	28	25
Mean litter size (day 0 lactation)	11.5[b]	13.3	13.4	12.8
Mean no. stillbirths:	0.50[c]	0.17	0.07	0.31
Number of viable males (day 0)	131	114	182	150
Number of viable females (day 0)	133	126	194	171
Sex ratio day 0 (% males)	49.6	47.5	48.4	46.7
Survival indices - Sexes pooled:				
Mean % pups surviving days 0-4 (pre-cull):	89.6	98.7	97.1	99.5
Mean % pups surviving days 4-21 (post-cull):	96.6	98.6	99.6	98.4

(continued)

[a]Control = Nos. 1731, 1741, 1740, 1746, 1758 and 1759 not pregnant. No. 1755 - no viable litter uterine implant observed at necropsy.

[b]See Appendix 7.6.6., Table F

[c]See Appendix 7.6.6., Table C.1

10 ppm = Nos. 2732, 2736, 2739, 2741, 2742, 2745, 2746, 2747, 2748, 2750, 2755, 2757 - not pregnant.

50 ppm = Nos. 3742 and 3758 - not pregnant.

500 ppm = Nos. 4740, 4742, 4753 and 4758 - not pregnant. No. 4738 - no viable litter - 10 uterine implants observed at necropsy.

See Appendices 7.6.6., 7.10.7., and 7.10.9.2

Reviewed by: Sanford W. Bigelow, Ph.D. 3/28/88
Section VI, Toxicology Branch (TS-769C)
Secondary reviewer: Judith W. Hauswirth, Ph.D.
Section VI, Toxicology Branch (TS-769C) Judith W. Hauswirth 3/28/88

DATA EVALUATION REPORT

I. **SUMMARY:**

STUDY TYPE: oncogenicity - mouse (83-2) CASWELL NO: 63

ACCESSION NUMBER: MRID NO.: 404313-02

TEST MATERIAL: Atrazine

SYNONYMS: 2-Chloro-4-ethylamino-6-isopropylamino-s-triazine

STUDY NUMBER: 842120

SPONSOR: CIBA-GEIGY Corp., Agricultural Division, P.O. Box 18300 Greensboro, NC 27419 Thomas Parshley, Regulatory Specialist (919) 292-7100 X7207

TESTING FACILITY: Division of Toxicology/Pathology, Ciba-Geigy Corp., Summit, NJ 07901

TITLE OF REPORT: Atrazine - technical: 91-week oral carcinogenicity study in mice.

AUTHORS: J.R. Hazelette, Ph.D. and J.D. Green, Ph.D.

REPORT ISSUED: October 30, 1987

CONCLUSIONS: Atrazine was not oncogenic to the CD-1 strain of mouse under the conditions of this assay.

NOEL = 300 ppm (45.0 mg/kg)
LEL = 1500 ppm (225.0 mg/kg) based upon the effects found in male and female mice. The NOEL and the LEL were determined on the following bases. The LEL of 1500 ppm was based upon decreases of 23.5% and 11.0% in mean body weight gain found at 91 weeks in male and female mice, respectively. Also, an increase in the incidence of cardiac thrombi was found in female mice in the 1500 ppm exposure group. None of the above effects were found at 300 ppm, thus the NOEL for atrazine in mice was set at 300 ppm.

At the highest exposure level, 3000 ppm, atrazine exposure in both sexes of mice caused:

1) a decrease in the mean body weight gain at 12 and 91 weeks,

2) a decrease in food consumption rates,

3) an increase in the incidence of cardiac thrombi,

4) a decrease in erythrocyte count, hemoglobin concentration and hematocrit, and

in female mice only, atrazine exposure caused:

1) an increase in mortality,

2) a decrease in mean brain and kidney weights, and

3) decreased percentages of neutrophils and lymphocytes.

Classification: core-guideline: This classification is based on the fact that the methodology requirements established in the Pesticide Assessment Guidelines, Subdivision F §83-2 have been satisfied.

II. MATERIALS:

A. Test Compound: atrazine

Description: atrazine, technical-grade
Batch #: 841802
Purity: The purity of atrazine used in this study was not given.

B. Test Animals:

Species: Mouse
Strain: CD-1 [Crl: CD1 (ICR) BR]
Age: about 5 weeks
Weight (mean, in grams): females: 21.0 ± <20%
(at week 0) males: 26.8 ± <20%
Source: Charles River Laboratories, Kingston, NY

III. STUDY DESIGN:

A. Animal Assignment:

Animals were assigned randomly to the following test groups:

Table 1
Animal Assignment in this Study

Test Group	Dose in diet (ppm)	Main Study 91 weeks male	Main Study 91 weeks female	Least number of treatment weeks
1 Control-	-	59	60	91
2 Low1 (LDT1)	10	60	59	91
3 Low2 (LDT2)	300	60	60	91
4 Mid1 (MDT1)	1500	60	60	91
5 High (HDT)	3000	58	60	91

Upon arrival from Charles River Laboratories, all mice were quarantined for 2 weeks for observation prior to initiation of atrazine exposure. Atrazine feeding started October 31, 1984 and ended August 22, 1986.

B. Diet Preparation:

The diet containing atrazine was prepared within 2 weeks before initial atrazine exposure and thereafter, about every 3 weeks. Every lot of feed containing atrazine was used within 3 weeks of preparation. The feed was stored at room temperature, and on several occasions, at refrigerated temperatures. The feed was analyzed for concentration and/or homogeneity on weeks 5, 9, 13, 17, 21, 29, 33, 37, 41, 45, 49, 60, 68, 76, 84, 92 and 94. This analysis was performed in the Toxicology/Pathology Administration and Technical Operations Section (of Ciba-Geigy in Summit, NJ) prior to use.

Analytical results: These admixtures were reported to be stable for at least 40 days at room temperature. Analytical results state that storage at room temperature caused less than 10% variation in the stability, homogeneity or concentration of atrazine in the laboratory chow.

The drinking water (tap water) was analyzed periodically according to the standard operating procedure of the Safety Evaluation Facility and was found to contain no detectable levels of contaminants.

Feeding schedule: Animals received food (called Certified Purina Rodent Chow #5002) and water ad libitum throughout the 91 week study.

C. Statistics:

The following statistical procedures were utilized in analyzing the numerical data:

The Barlett's test was conducted for determining homogeneity in variances (presence of a normal distribution) between treatment groups. If the variance was found to be similar between groups by the above tests, Dunnett's tests were conducted to compare values of the control and treatment groups.

When outliers (or heterogeneous variances between groups) were identified, supplemental statistical analyses were performed. Examples of these supplemental statistical analyses were: (1) the use of an appropriate transformation of the data or (2) nonparametric tests. In addition, several test results that are known not to be distributed normally were analyzed with the use of nonparametric tests.

Concerning the pathology data sets, if their sample size was found adequate, these data were analyzed for each sex by the Fisher's exact test. Tumor incidences were analyzed by a time-adjusted analysis by Peto's method. Statistical differences for survival curves between treatment groups for each sex were examined by the use of the following statistical methods: (1) the generalized Wilcoxon test for equality, (2) the Mantel-Cox logrank test equality and test for linear trend, (3) nonparametric tests and (4) Kaplan-Meier estimates.

D. Quality Assurance:

A signed quality assurance statement was provided by a quality assurance inspector. According to the statement, the study was audited sixteen (16) times during the course of the study.

IV. METHODS AND RESULTS:

A. Clinical Observations:

Animals were inspected twice daily for mortality and once daily for general appearance, behavior and excreta.

Viral screens were performed on 6 male and 6 female mice taken randomly from the colony 2 weeks before atrazine feeding began. The presence of the following viruses were checked: minute virus, pneumonia virus, reovirus (type 3), hepatitis virus, K virus, murine encephalomyelitis virus, Sendai virus, lymphocytic choriomeningitis virus, adenovirus, ectromelia virus, polyoma virus and mycoplasma pulmonis.

Toxicity/mortality (survival) results: A total of 301 of the 596 mice used in the study died. For female mice fed 3000 ppm atrazine, there was a statistically significant decrease in survival whereas for males, atrazine exposure had no statistically significant effect on survival.

Table 2
Summary of Mortality
(taken from p. 41)

Sex:	Males					Females				
Group #:	1	2	3	4	5	1	2	3	4	5
Dose (ppm):	0	10	300	1500	3000	0	10	300	1500	3000
# of mice:	59[a]	60	60	60	58[b]	60	59[a]	60	60	60
Reason for sacrifice:										
Found dead:	20	18	16	18	17	27	32	27	29	39
Sacrificed moribund:	4	9[c]	8	3	6	7	4	7	4	6
Terminal sacrifice:	35	33	36	39	35	26	23	26	27	15
% survival at term:	59	55	60	65	60	43	39	43	45	25[d]

[a] Two mice were deleted due to misidentification.
[b] Two mice in group 5 (3000 ppm) were mis-sexed, and therefore their data results were deleted from reporting.
[c] One mouse in Group 2 (10 ppm) escaped from its cage and was sacrificed.
[d] $p < 0.05$, generated from a survival analysis with the use of Mantel-Cox Logrank test.

Clinical Results: There were no treatment-related clinical signs observed during the study. Clinical signs most frequently observed were: lesions, alopecia, scabs, perineal stains, fur stains and dermatitis in all groups of mice. No treatment-related changes in the incidence of palpable masses occurred during the study.

B. Body weight:

All animals were weighed weekly for weeks 1-12, biweekly during weeks 14-25, and at 4 week intervals thereafter.

Results: Dose-related reductions were observed at weeks 12 and 91 in mean body weight gain (either % decrease or % gain) in both sexes of mice who were exposed to chow containing 1500 ppm or 3000 ppm atrazine. Table 3 shows the changes in mean body weight gain at weeks 12 and 91 in mice fed atrazine. At week 12 males exhibited decreases of 14.2% and 10.4% in mean body weight gain in the 10 ppm and 300 ppm exposure groups, respectively. This effect appears to be transient in nature because it is not observed at 91 weeks at the same magnitude.

Table 3
Mean Body Weight Gain Changes in Mice Fed Atrazine
(taken from Table 8.3)

Dose (ppm)	Week 12 Gain (g)	Week 12 % Decrease[a] (increase)	Week 12 % Gain[b]	Week 91 Gain (g)	Week 91 % Decrease[a] (increase)	Week 91 % Gain[b]
			Males			
0	10.6	NA	39.1	11.5	NA	43.7
10	9.1	14.2	37.1	13.3	(15.6)	49.4
300	9.5	10.4	36.1	10.4	9.6	39.5
1500	7.1	33.0	26.7[d]	8.8	23.5	32.9[c]
3000	6.3	40.6	23.8[d]	8.7	24.4	33.1[c]
			Females			
0	9.1	NA	43.7	13.6	NA	68.8
10	9.3	(2.2)	44.5	13.0	4.4	64.6
300	8.5	6.6	40.5	12.6	7.4	64.1
1500	7.8	14.3	37.9[c]	12.1	11.0	61.7
3000	8.0	12.1	37.6[c]	7.0	48.5	33.3[d]

[a] The reviewer calculated body weight gain by the following formula:

$$\% \text{ Decrease (increase)} = 100 - \frac{\text{Mean body weight gain (test group)}}{\text{Mean body weight gain (control)}} \times 100$$

where: Mean body weight gain = body weight (g) for wk 12 (or wk 91) - body weight (g) for wk 0

[b] The authors of the study calculated % body weight gain by the following formula:

$$\% \text{ Gain} = \frac{\text{Body weight (week 12 or 91)}}{\text{Body weight (week 0)}} \times 100$$

[c] $p = \leq 0.05$ and [d] $p = \leq 0.01$, significantly different from control group when compared by the use of the two-tailed Dunnett t-test performed on the raw data.
NA = Not applicable

C. Food consumption and compound intake:

Food consumption was determined in all animals on a weekly basis during weeks 1-12, biweekly during weeks 14-25 and at 4 week intervals thereafter. Mean daily diet consumption were calculated from these data. The intake of atrazine was calculated and is reported below. Efficiency and atrazine intake were calculated from the consumption and body weight gain data.

Water consumption was measured in all animals on weeks 1, 2, 52, 53, 90 and 91. [Subdivision F (§83-2, section 7 part vi) states that water consumption should be monitored weekly during the first 13 weeks of a study and then at 4 week intervals thereafter (p. 121).]

Food consumption results: Treatment-related reductions in mean food consumption were observed in Group 4 males (1500 ppm) and Group 5 males and females (3000 ppm). Statistically significant reductions in mean water intake were noted primarily in mice fed 1500 or 3000 ppm atrazine. Reductions in mean food consumption correlated with similar reductions in mean body weight and mean body weight gain. These reduction were sporadic (occurred only in certain weeks during the study) and were not related to the dose of atrazine. No statistically significant reductions in mean food consumption were seen in mice fed chow containing 10 ppm atrazine.

Table 4
Dietary Intake of Atrazine
(taken from p. 19)

		Dietary Concentration	Mean Daily Dose (mg/kg)[a]	Range (mg/kg/day)
Males:				
Group #	2	10	1.4	1.2 - 2.0
	3	300	38.4	35.7 - 58.3
	4	1500	194.0	184.3 - 293.3
	5	3000	385.7	364.3 - 541.6
Females:				
Group #	2	10	1.6	1.4 - 2.3
	3	300	47.9	41.1 - 73.1
	4	1500	246.9	215.9 - 363.3
	5	3000	482.7	420.8 - 660.6

[a] The group mean daily dose was calculated for each study week as follows:

$$\text{Group Mean Daily Dose (mg/kg)} = \frac{\text{Group Mean Food Consumption (g/mouse/day)} \times \text{Atrazine Conc. (mg/kg)}}{\text{Mean Group Mid-Period Body Weight (g)}}$$

Conclusion: On the basis of a daily dose of mg/kg, famale mice fed 300, 1500, or 3000 ppm atrazine received about a 25% higher daily dose of atrazine than male mice in the corresponding exposure group.

D. Ophthalmological examination:

Ophthalmological examinations were performed prior to the study on all male and all female rats on weeks 26, 52, 78 and 90.

Results and conclusions: No treatment-related ophthalmic changes were observed during this study. Corneal opacities and lenticular cataracts were the most frequent observations and occurred with similar incidence in both control and treated groups of mice (see table below). Most animals with ocular changes noted early in the study (i.e., examined at weeks 26 or 52) had no ocular changes when examined at weeks 78 and 90.

Table 5
Summary Incidence of Ocular Findings at 90 Weeks
(taken from p. 2709)

Sex:	Males					Females				
Group #:	1	2	3	4	5	1	2	3	4	5
Dose (ppm):	0	10	300	1500	3000	0	10	300	1500	3000
# of mice:	38	36	37	43	37	28	25	27	31	15
Ocular Findings:										
Cornea: opacity	14	9	10	17	12	9	9	4	10	2
Lens: catract	22	17	20	17	24	23	18	24	25	15
Adnexa:										
blepharitis	2	3	3	2	3					
Iris:										
ectopic pupil	1		1	1	2					
Phthisis bulbi	1									

E. Hematology:

Blood was collected from all animals on days 362, 544 and 639 for hematology and clinical analysis from all animals. Blood smears were obtained during weeks 52 and 78 from the first 20 animals for each sex in the 0 ppm and 3000 ppm atrazine groups. In addition, all animals who died or who were sacrificed in moribund conditon had blood smears taken. The CHECKED (X) parameters were examined.

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (Hb)*		Mean corpuscular Hb (MCH)
X	Leukocyte count (WBC)*	X	Mean corpuscular Hb conc.(MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)
	Platelet count*+		Reticulocyte count
	Plateletcrit		Mean platelet volume
	Platelet dist. width		Red cell dist. width
	Blood clotting msrmts.		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Required for subchronic and chronic studies
\+ Not required for oncogenicity studies

Results and conclusions: At the termination of the study, statistically significant reductions in mean erythroid variables (erythrocyte count, hematocrit and hemoglobin) were observed in Groups 4 (1500 ppm) and 5 (3000 ppm) males and Group 5 females. The authors concluded that these erythroid effects were secondary to decreased body weight, food consumption and/or water consumption. These results are summarized in Table 6.

Other hematological effects were observed. Group 5 females (3000 ppm) had reduced mean neutrophil percentage and elevated lymphocyte percentage when compared to control mice (Table 6). These elevated blood cell levels may have been caused by by illness, although the authors did not fully elaborate on these results.

A few male and female blood samples in those mice who survived to terminal necropsy were not analyzed, regardless, the number of samples only amounted to 1 per group (compare Tables 2 and 9).

Table 6
Selected Hematological Parameters in Mice at 639 Days
(taken from Table 8.7)

Group #:	1	2	3	4	5
Dose (ppm):	0	10	300	1500	3000
		# of mice examined			
males:	34	33	35	39	34
females:	26[c]	23	26[c]	26	15
Parameter:					
			Males		
RBC	7.68	7.47	7.48	6.69[b]	6.33[b]
Hb	14.71	14.02	14.07	12.86[a]	12.52[b]
HCT (%)	45.24	43.21	43.66	39.74[a]	38.62[b]
			Females		
RBC	6.64	7.34	6.36	6.29	5.54[a]
Hb	13.25	14.33	12.62	12.58	11.22[a]
HCT (%)	41.04	43.96	39.04	38.62	34.80[a]
Neuts %[c]	39.52	37.22	40.84	49.23	56.40[a]
Lymphs %[c]	58.32	60.61	57.92	48.54	42.33[a]

[a] p = <0.05, [b] p = <0.01, significantly different from control group when compared by the use of the two-tailed Dunnett t-test performed on the raw data.
[c] = For neutrophil % and lymphocyte % tests, 25 female control mice and 25 female mice in the 300 ppm exposure group were examined.

F. Sacrifice, Gross Pathology and Histopathology:

All animals were fasted overnight prior to terminal necropsy. The 301 animals that died in the course of the study and those mice who were sacrificed on schedule were examined for gross pathological and histological changes. Terminal necropsies began August 1, 1986 and ended August 22, 1986 on weeks 92-95 of the study. Necropsies were also performed on the animals who had died during the course of the study. Microscopic examinations were performed on all specificed tissues and gross lesions from all animals in each group, regardless whether the animal was found dead, sacrificed moribund, or after scheduled necropsy.

The CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed to determine the organ weight.

	Digestive system		Cardiovascular		Neurological
X	Tongue	X	Aorta*	XX	Brain*+
X	Salivary glands*	X	Heart*	X	Periph. nerve (sciatic)*#
X	Esophagus*	X	Bone marrow*#	X	Spinal cord (3 levels)*#
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	X	Spleen	X	Eyes (optic n.)*#
X	Jejunum*	X	Thymus*		Glandular
X	Ileum*		Urogenital	XX	Adrenal gland*
X	Cecum*	XX	Kidneys*+		Exorbital lacrimal gland#
X	Colon*	X	Bladder*	X	Mammary gland*#
X	Rectum*	XX	Testes*+	X	Parathyroids*++
XX	Liver*+	X	Epididymides	X	Thyroids*++
X	Gall bladder*#	X	Prostate		Other tissues
X	Pancreas*	X	Seminal vesicle		Bone (femur)*#
	Respiratory	X	Ovaries*+	X	Skeletal muscle(thigh)*#
X	Trachea*#	X	Uterus*	X	Skin*#
X	Lung*		Cervix	X	All gross lesions and masses*
	Nose^		Fallopian tubes		
X	Pharynx^	X	Vagina		
X	Larynx^				

* Required for subchronic and chronic studies.
^ Required for chronic inhalation.
\# In subchronic studies, examined and preserved only if indicated by signs of toxicity or target organ involvement.
\+ Organ weight required in subchronic and chronic studies.
++ Organ weight required for non-rodent studies.

1. Organ weight: Organ weights were determined for the liver, kidneys, testes, brain and adrenals in all animals in all exposure groups at weeks 92-95 during terminal necropsy. Organ weights were not recorded for animals found dead or sacrificed moribund. Only 26 of the 36 surviving females had their livers weighed whereas 34 of 35 surviving males had their livers weighed, and therefore, not all of the mice who were fed atrazine had their organs examined.

   Organ weight results: Few organ weight changes were observed in this study. Mice fed 3000 ppm exhibited decreased mean weight in the following tissues: brain (females and males) and kidneys (females). Organ weight increases were found in mean brain to body weight ratio in females. Table 7 shows the organ weights of the brain, adrenal gland, kidney, liver and testes as well as the organ weight ration to whole body weight.

Table 7
Selected Organ Weights and Weight Ratios at 91 Weeks
(% body weight ratios in parentheses)
(taken from Table 8.8)

Group #:	1	2	3	4	5
Dose (ppm):	0	10	300	1500	3000
Organ (in grams):					
			Males		
Brain	0.55 (1.55)	0.50 (1.45)	0.50 (1.55)	0.49 (1.55)	0.48[b] (1.52)
Adrenal	0.007 (0.02)	0.008 (0.02)	0.007 (0.02)	0.007 (0.02)	0.007 (0.02)
Kidney	0.65 (1.96)	0.67 (1.93)	0.64 (1.95)	0.62 (1.96)	0.60 (1.90)
Liver	1.61 (4.81)	1.62 (4.75)	1.53 (4.69)	1.54 (4.85)	1.53 (4.83)
Testes	0.32 (0.96)	0.32 (0.94)	0.33 (1.01)	0.31 (1.00)	0.29 (0.94)
			Females		
Brain	0.52 (1.73)	0.53 (1.78)	0.52 (1.76)	0.51 (1.83)	0.49[a] (2.01)[b]
Adrenal	0.01 (0.04)	0.01 (0.03)	0.01 (0.03)	0.01 (0.03)	0.01 (0.05)
Kidney	0.49 (1.61)	0.48 (1.62)	0.47 (1.60)	0.44 (1.59)	0.41[b] (1.70)
Liver	1.60 (5.26)	1.48 (4.93)	1.49 (5.02)	1.54 (5.50)	1.41 (5.68)

[a] p = <0.05, [b] p = <0.01, significantly differnet from control group when compared by the use of the two-tailed Dunnett t-test performed on the raw data.

2. Gross pathology results: Several gross observations were noted in the mice fed higher levels (i.e., 1500 ppm and/or 3000 ppm) of atrazine. These observations were: enlarged atrium (or atria) of the heart, tan-colored lesions of the heart and pallid color of the kidney(s). The observation of enlarged atria of the heart appears to be dose-related although it occurs at a low incidence. The incidences of these gross lesions are illustrated in the Table 8 below.

Table 8
Summary Incidence of Gross Lesions Observed in this Study
(taken from Table 9.6.3)

Sex:	Male Mice					Female Mice				
Group #:	1	2	3	4	5	1	2	3	4	5
Dose (ppm):	0	10	300	1500	3000	0	10	300	1500	3000
Total # of mice:	59	60	60	58	58	60	59	60	60	60
Organ or Site:										
Heart, l. atrium:										
enlarged	1			4	4			1	4	8
Lesion, tan				2	2				3	3
Heart, r. atrium:										
enlarged	1			1	2				1	13
Kidney, pallid color	2		3	2		3	4	3	10	3

3. Histopathology results:

a) Non-neoplastic lesions: As seen after the terminal necropsy, dose-related cardiac thrombi (primarily in the atria) were seen in male mice receiving 1500 ppm and female mice fed 3000 ppm atrazine. As shown in Table 9, cardiac thrombi were observed primarily in those animals who had died or were killed in the course of the study.

The authors attributed the majority of the unscheduled deaths to spontaneously-occurring renal amyloidosis (p. 24). However, the incidence of cardiac thrombi in mice with unscheduled deaths and who were sacrificed moribund is statistically significant from control mice (this group of mice is referred by the authors as "early deaths" -- this term is adopted in this review). Statistically significance was not found in any group of mice regarding the incidence of renal amyloidosis in any group of mice. Table 9 shows that the incidence of cardiac thrombi in female mice treated with 1500 ppm or 3000 ppm was higher in the "early death" group of female mice than the corresponding female mice who survived to terminal necropsy.

Other statistically significant amyloid lesions occurred in exposed groups but were termed "sporadic" by the authors. The following amyloid lesions were observed:

1) A statistically significant increase in incidence of amyloidosis of the liver and the adrenal gland were found in female mice fed 300 ppm atrazine.

2) Likewise, thyroid amyloidosis was observed the "early death" group of female mice who ate chow containing 10 ppm atrazine.

3) Lymph node amyloidosis was observed in the "early death" group of female mice who were fed 1500 ppm atrazine.

Table 9
Summary Incidence of Cardiac Thrombi Observed in this Study
(taken from p. 2749)

Sex:	Male Mice					Female Mice				
Group #:	1	2	3	4	5	1	2	3	4	5
Dose (ppm):	0	10	300	1500	3000	0	10	300	1500	3000
"Early death" group	3/24	5/27	3/24	7/21	9/23[a]	3/34	4/36	1/34	11/33[b]	24/45[a]
Mice surviving to terminal sacrifice	0/35	1/33	0/36	0/39	0/35	0/26	0/23	1/26	0/27	2/15
All mice	3/59	6/60	3/60	7/60	9/58	3/60	4/59	2/60	11/60[a]	26/60[c]

[a] = $p < 0.05$, [b] = $p < 0.01$, [c] = $p < 0.001$, significantly different from control (by Fisher's exact test).

Table 10
Summary Incidence of Renal Amyloid Lesions Observed in this Study
(taken from Tables 9.6.1.1, 9.6.1.2, 9.6.1.3)

Sex:	Male Mice					Female Mice				
Group #:	1	2	3	4	5	1	2	3	4	5
Dose (ppm):	0	10	300	1500	3000	0	10	300	1500	3000
"Early death" group	9/24	10/27	6/24	8/21	6/23	18/34	22/36	23/34	20/32	25/45
Mice surviving to terminal sacrifice	5/35	2/33	4/36	3/39	8/35	8/26	5/23	7/26	5/27	0/15
All mice	16/59	9/60	14/60	14/60	18/58	28/60	31/59	31/60	25/60	29/60

Table 11
Summary Incidence of Adrenal Amyloid Lesions Observed in this Study
(taken from Tables 9.6.1.1, 9.6.1.2, 9.6.1.3)

Sex:	Male Mice					Female Mice				
Group #:	1	2	3	4	5	1	2	3	4	5
Dose (ppm):	0	10	300	1500	3000	0	10	300	1500	3000
"Early deaths" group	8/24	8/27	6/24	11/20	9/22	18/34	24/35	24/33	20/32	30/44
Mice surviving to terminal sacrifice	3/32	1/32	2/35	0/37	8/32	5/26	2/23	9/25	6/27	0/15
All mice	11/56	9/59	8/59	11/57	17/54	23/60	26/58	33/58[a]	26/59	30/59

[a] = $p < 0.05$, significantly different from control (by Fisher's exact test).

Table 12
Summary Incidence of Hepatic Amyloid Lesions Observed in this Study
(taken from Tables 9.6.1.1, 9.6.1.2, 9.6.1.3)

Sex:	Male Mice					Female Mice				
Group #:	1	2	3	4	5	1	2	3	4	5
Dose (ppm):	0	10	300	1500	3000	0	10	300	1500	3000
"Early deaths" group	7/24	7/27	4/24	10/20	9/23	17/34	23/36	23/34	19/33	29/45
Mice surviving to terminal sacrifice	2/35	1/33	2/35	0/39	4/34	4/26	2/23	6/25	5/27	0/15
All mice	8/59	7/60	5/60	10/59	10/58	20/60	25/59	30/60[a]	22/60	29/60

[a] = $p < 0.05$, significantly different from control (by Fisher's exact test).

Table 13
Summary Incidence of Lymph Node Amyloid Lesions Observed in this Study
(taken from Tables 9.6.1.1, 9.6.1.2, 9.6.1.3)

Sex:	Male Mice					Female Mice				
Group #:	1	2	3	4	5	1	2	3	4	5
Dose (ppm):	0	10	300	1500	3000	0	10	300	1500	3000
"Early deaths" group	3/22	0/25	0/23	0/16	3/21	5/34	8/32	10/32	11/31[a]	8/41
Mice surviving to terminal sacrifice	1/35	1/31	2/35	0/36	1/33	3/26	3/23	3/26	3/26	0/14
All mice	4/57	1/56	2/58	0/52	4/54	8/60	11/55	13/58	14/57	8/55

[a] = $p < 0.05$, significantly different from control (by Fisher's exact test).

Table 14
Summary Incidence of Thyroid Gland Amyloid Lesions Observed in this Study
(taken from Tables 9.6.1.1, 9.6.1.2, 9.6.1.3)

Sex:	Male Mice					Female Mice				
Group #:	1	2	3	4	5	1	2	3	4	5
Dose (ppm):	0	10	300	1500	3000	0	10	300	1500	3000
"Early death" group	8/24	8/25	5/23	9/21	10/22	17/34	25/34[a]	23/34	20/33	29/45
Mice surviving to terminal sacrifice	2/35	1/33	2/35	0/39	4/34	4/26	2/23	6/25	5/27	0/15
All mice	10/59	9/58	7/58	9/60	14/56	21/60	27/57	29/59	25/60	29/60

[a] = $p < 0.05$, significantly different from control (by Fisher's exact test).

b) Neoplastic lesions: Overall, atrazine exposure did not cause a dose-related increased incidence of neoplasms in these mice in this study.

Histological evaluation of palpable masses were performed. Of the palpable masses examined, 3 female mice were found to have developed mammary adenocarcinomas (one mouse in the control group and 2 mice fed chow containing 3000 ppm atrazine). One female in the 10 ppm exposure group developed a fibroma and one female in the 300 ppm group developed malignant lymphoma. In male mice, two developed fibrosarcoma in the 10 ppm exposure group. One male mouse in the group fed 1500 ppm atrazine was determined to have a hemangiosarcoma (a malignancy formed by the proliferation of endothelial and fibroblastic tissue). These neoplasms were found after histological examination of these palpable masses in these mice; some of these tumors are listed in the tumor incidence tables below.

As shown on Table 15, in male mice fed 10 ppm atrazine, a statistically significant increase in the incidence of hepatocellular adenomas was observed, yet no statistically significant increase in incidence of this type of tumor was observed in groups of mice fed higher levels of atrazine (i.e., 300 ppm, 1500 ppm or 3000 ppm). This effect is not dose-related.

No statistically significant increases in incidence were found for the following types of neoplasms: mammary adenocarcinomas, adrenal adenomas, pulmonary adenomas and malignant lymphomas. The incidences for these tumors are listed in the tables below.

Table 15
Summary Incidence of Hepatocellular Adenomas Observed in this Study
(taken from Tables 9.6.1.1, 9.6.1.2, 9.6.1.3)

Sex:	Male Mice					Female Mice				
Group #:	1	2	3	4	5	1	2	3	4	5
Dose (ppm):	0	10	300	1500	3000	0	10	300	1500	3000
"Early death" group	0/24	5/27[a]	0/24	1/20	0/23	0/34	0/36	0/34	0/33	0/45
Mice surviving to terminal sacrifice	10/35	8/33	6/36	3/39	1/35	1/26	0/23	0/26	0/27	0/15
All mice	10/59	13/60	6/60	4/59	1/58	1/60	0/59	0/60	0/60	0/60

[a] = $p < 0.05$, significantly different from control (by Fisher's exact test).

Table 16
Summary Incidence of Mammary Adenocarcinomas Observed in this Study
(taken from Tables 9.6.1.1, 9.6.1.2, 9.6.1.3)

Sex:	Male Mice					Female Mice				
Group #:	1	2	3	4	5	1	2	3	4	5
Dose (ppm):	0	10	300	1500	3000	0	10	300	1500	3000
"Early death" group	0/13	0/9	0/13	0/14	C/9	1/33	0/35	0/33	0/33	2/44
Mice surviving to terminal sacrifice	0/18	0/12	0/19	0/22	0/20	0/26	0/23	1/25	0/27	0/15
All mice	0/31	0/21	0/32	0/36	0/29	1/59	0/59	1/58	0/60	2/59

Table 17
Summary Incidence of Adrenal Adenomas Observed in this Study
(taken from Tables 9.6.1.1, 9.6.1.2, 9.6.1.3)

Sex:	Male Mice					Female Mice				
Group #:	1	2	3	4	5	1	2	3	4	5
Dose (ppm):	0	10	300	1500	3000	0	10	300	1500	3000
"Early deaths" group[a]	0/24	0/27	0/24	0/20	0/22	0/34	0/35	0/33	0/32	0/44
Mice surviving to terminal sacrifice	3/32	0/32	3/35	4/37	3/32	0/26	0/23	0/25	0/27	0/15
All mice	3/56	0/59	3/59	4/57	3/54	0/60	0/58	0/58	0/59	0/59

[a] = The numerator of these incidence values in this row were calculated by subtracting the tumor incidence in those mice who survived until terminal sacrifice from all of the mice studied (e.g., for Group 1 males, 3/56 - 3/32 = 0/24)

Table 18
Summary Incidence of Pulmonary Adenomas Observed in this Study
(taken from Tables 9.6.1.1, 9.6.1.2, 9.6.1.3)

Sex:	Male Mice					Female Mice				
Group #:	1	2	3	4	5	1	2	3	4	5
Dose (ppm):	0	10	300	1500	3000	0	10	300	1500	3000
"Early deaths" group	1/24	1/27	1/24	0/21	1/23	1/34	1/36	1/34	1/33	1/45
Mice surviving to terminal sacrifice	3/35	3/33	3/36	5/39	6/35	0/26	0/23	1/26	2/27	1/15
All mice	4/59	4/60	4/60	5/60	7/58	1/60	1/59	2/60	3/60	2/60

Table 19
Summary Incidence of Malignant Lymphoma Observed in this Study
(taken from Tables 9.6.1.1, 9.6.1.2, 9.6.1.3)

Sex:	Male Mice					Female Mice				
Group #:	1	2	3	4	5	1	2	3	4	5
Dose (ppm):	0	10	300	1500	3000	0	10	300	1500	3000
"Early deaths" group	2/24	3/27	5/24	0/21	1/23	6/34	5/36	5/34	3/33	3/45
Mice surviving to terminal sacrifice	2/35	5/33	4/36	3/39	3/35	13/26	9/23	11/26	9/27	4/15
All mice	4/59	8/60	9/60	3/60	4/58	19/60	14/59	16/60	12/60	7/60

V. **DISCUSSION:**

Atrazine exposure did not cause a dose-related increase in the incidence of neoplasms in the CD-1 strain of mice in this study. No dose-related effects are seen for macroscopic gross lesions or ocular changes in either sex during the 91-week atrazine feeding study.

The NOEL and the LEL are determined on the following bases. The LEL is set at 1500 ppm based upon decreases of 23.5% and 11.0% in mean body weight gain found at 91 weeks in male and female mice, respectively. Also, an increase in the incidence of cardiac thrombi is found in female mice in the 1500 ppm exposure group. None of the above effects are found at 300 ppm, thus the NOEL is set at 300 ppm.

This oncogenicity study shows that there are dose-related effects of atrazine in mice fed chow containing 1500 ppm or 3000 ppm atrazine. The dose-related effects are the production of cardiac thrombi, a decrease in the mean body weight gain at 12 and 91 weeks during the study, and decreases in erythrocyte count, hematocrit and hemoglobin concentration. An increase in the incidence of cardiac thrombi is found in female in the 1500 ppm and 3000 ppm exposure groups. In addition to amyloidosis, cardiac thrombi contributed to the deaths of the group of mice who did not survive to terminal sacrifice (this group of mice are termed as "early death" mice). This assertion is based on the observation that a statistically significant increased incidence of cardiac thrombi is found in "early death" mice whereas no statistically significant increase in incidence of cardiac thrombi is found in the group of mice who survived to terminal sacrifice in the same exposure group. These responses are the only dose-related effects observed in these mice in this study.

Female mice in the 3000 ppm exposure group recieved almost twice the dietary intake levels of atrazine when compared to male mice in the 3000 ppm exposure group. This observation may explain the 25% survival of female mice and 60% survival of male mice in the 3000 ppm exposure group.

At the highest exposure level, 3000 ppm, atrazine exposure in both sexes of mice caused:

1) a decrease in the mean body weight gain at 12 and 91 weeks,

2) a decrease in food consumption rates,

3) an increase in the incidence of cardiac thrombi,

4) a decrease in erythrocyte count, hemoglobin concentration and hematocrit, and

in female mice only:

1) an increase in mortality,

2) a decrease in mean brain and kidney weights, and

3) decreased percentages of neutrophils and lymphocytes.

Both the 1500 ppm and the 3000 ppm atrazine exposure levels are deemed sufficient doses to cause an adequate level of toxicity in male and female mice because:

1) the high percentage of mortality at 91 weeks (75%) in female mice in the 3000 ppm atrazine exposure group,

2) decreased mean body weight gain at 91 weeks in female mice (48.5%) and in male mice (24.4%) fed chow containing 3000 ppm atrazine,

3) a 23.5% decrease in mean body weight gain in male mice and an 11.0% decrease in female mice in the 1500 ppm atrazine exposure group at 91 weeks, and

4) at 12 weeks a 33.0% decrease in mean body weight gain in male mice and a corresponding 14.3% decrease in female mice in the 1500 ppm exposure group.

This study was well conducted and has been deemed sufficient quality to determine the oncogenic potential of atrazine. This study should be given the core classification of "guideline" because the methodology requirements established in the Pesticide Assessment Guidelines, Subdivision F §83-2 have been satisfied.

Irving Mauer 4-11-88 006718

Reviewed By: Irving Mauer, Ph.D. TB Project No.: 8-0320
Section VI, Toxicology Branch (TS-769C) Date:
Secondary Reviewer: Judith W. Hauswirth, Ph.D., Head
Section VI, Toxicology Branch (TS-769C)
Judith W. Hauswirth 4/20/88

TOXICOLOGY BRANCH: DATA REVIEW

Chemical: Atrazine Caswell No.: 063
TOX Chem No.: 080803

Study Type: Chronic (52-Week) Feeding - Dog

Citation: Atrazine Technical - 52-Week Oral Feeding in Dogs (MIN 852008)

Accession No.: 404313-01 (3 volumes)

Sponsor: Ciba-Geigy, Corporation, Greensboro, NC

Testing Lab.: Ciba-Geigy, Division of Toxicology/Pathology
Summit, NJ

Study No.: 852008 (Tox./Path. Rpt. No. 87048)

Study Date: October 27, 1987

TB Conclusions/Evaluation:

CORE MINIMUM DATA. Although the mid-dose (150 ppm) was proposed by the testing laboratory as the NOEL, a number of minimal cardiac changes were found in a few animals at this intermediate dose. Hence, TB regards the LDT (15 ppm) as the NOEL, until more exactly defined between 15 and 150 ppm.

Dietary doses administered: 0, 15, 150, and 1000 ppm.

Intake equivalent: mg/kg/day

Male - 0, 0.48, 4.97, and 33.65

Female - 0, 0.48, 4.97, and 33.80

DETAILED REVIEW

Test Article:

Atrazine technical (Batch FL 850612); nature and purity not stated here (but reported elsewhere as 97 percent); mixed in canine feed (Purina No. 5007) for oral feeding. Samples of the admixture were monitored at regular intervals for contaminants, stability, homogeneity, and atrazine concentration.

Procedures:

Purebred 5-month-old male and female beagle dogs (Marshall Farms, North Rose, NY) were acclimated to the laboratory for 1 month, then assigned randomly to four groups, which received feed containing 0 (feed only, six animals/sex), 15 ppm (four animals/sex), 150 ppm (four/sex), and 1000 ppm (six animals/sex) test article.

The animals were observed daily, and body weight and food consumption determined pretreatment, and weekly during the first 13 weeks of treatment, then monthly thereafter. Auditory and ophthalmoscopic (by Fison Indirect Ophthalmoscope) examinations were performed during the pretreatment period and quarterly during treatment, as were electrocardiographic (EKG) tracings (10 leads). Seven hematological parameters (hemoglobin, hematocrit, rbc, wbc, differential, platelets, and prothrombin time) were also measured during the pretreatment period and quarterly thereafter, in addition to reticulocytes and Heinz bodies. Fifteen serum chemistry values (BUN, creat, SGOT, SGPT, alk phos, glu, tot bili, tot chol, inorg phos, Na, K, Ca, Cl^-, tot prot, alb, glob, and A/G ratio) including CPK and LDH, and nine urinalysis values (spec grav, pH, prot, glu, bili, urobili, ket, occ blood and micro) were also determined according to the same sampling schedule. All surviving animals were necropsied during week 53; complete necropsies were also carried out on any animals that died during the study period.

A complete roster of tissues, according to Test Guideline directives, was fixed in 10% neutral buffered formalin for microscopic examination:

Adrenal (2)
All Gross Lesions incl. Tissue Masses
Aorta
Brain:
 cerebrum
 cerebellum
 medulla/pons
Kidney (2)
[Larynx/Pharynx]
Liver
Lung
Lymph node:
 Axillary
 Mesenteric
Mammary gland (F)
Salivary Gland
Skin
Spinal Cord:
 cervical
 lumbar
 thoracic
Spleen
Sternum w/marrow

Cecum	Muscle, thigh	Stomach
Colon	Nerve, sciatic	Testis w/epididymis (2)
Duodenum	Ovary (2)	Thymus, when present
Esophagus	Pancreas	Thyroid w/parathyroid (2)
Eyes w/optic nerve	Pituitary	Tongue
[Femur w/joint]	Prostate	Trachea
Gallbladder	Rectum	Urinary Bladder
Heart	[Rib at costochondral	Uterus
Ileum	joint]	Vagina
Jejunum		[Vertebra]

Absolute organ weights (brain, heart, kidney, liver, ovary, pituitary, spleen, testis with epididymis, and thyroid/parathyroid) were determined, and relative weights calculated using a computer system. Gross pathology data were initially recorded manually, but later entered into the NO3 pathology data base. A detailed examination of the hearts of all animals sacrificed at study termination was performed, including measurement of left and right ventricular wall thickness, in order to augment the description of the nature and severity of any cardiac lesions. [NB: Cardiac toxicity was the subject of a flagging statement in the final report of this study (Volume 1, page 4 of 1405).]

Data on body and organ weights, feed consumption, clinical laboratory values, and EKG tracings were stored in the Beckman toxicology system data base (TOXSYS) in the IBM 4361 mainframe computer, and analyzed separately for each sex by a two-tailed Dunnett's multiple comparisons test ($p < 0.05$ and < 0.01) at each time point during treatment, in order to detect any differences between each treatment mean and control. During the pretreatment period, an F-test for testing equality of all treatment group means was performed. Nonparametric versions of these procedures (based on rank rather than numerical values) were employed on parameters not normally distributed (e.g., highly skewed). Supplemental statistical analyses were necessary where the significant deviations were detected via diagnostic procedures. These included data transformation, nonparametric tests, and tests without assuming homogeneity of group variances.

Results:

Chemical Analysis/Dosimetry

Routine (monthly) chemical analysis of feed admixtures indicated that the concentrations of the test material were within ± 10 percent of target concentrations (92 to 107 percent, Appendix 9.8). The admixtures were stated to be stable for at least 21 days at room temperature (92 percent of target concentrations for the "15 ppm" test formulation, Appendix 9.7). (NB: The same tabulation indicates, however, a 99 percent target stability for the high-dose admixture, 1000 ppm.) Analysis of

multiple subsamples during week 1 of treatment revealed atrazine was homogeneously distributed throughout the feed admixtures (relative standard deviations were 2, 1.3, and 1.7 percent, respectively, for the three dosage groups: 15, 150, and 1000 ppm, Appendix 9.9).

Based on feed consumption and average-group mid-period body weight (Table 8.4, Appendix 9.1.4, discussed below), the daily doses and ranges (from Table 8.3) were calculated as follows:

Sex	Group	Dietary Concentration (ppm)	Mean Daily Dose (mg/kg/day)	Range (mg/kg/day)
M	2	15	0.48	0.4 - 0.6
	3	150	4.97	4.3 - 5.7
	4	1000	33.65	20.1 - 38.0
F	2	15	0.48	0.4 - 0.6
	3	150	4.97	4.2 - 6.0
	4	1000	33.80	23.1 - 39.3

Mortality/Clinical Signs

Three animals had to be sacrificed during the study in a moribund condition: one 150 ppm male (14 M) on day 75; one 1000 ppm female (39 F) on day 113; and one 1000 ppm male (16 M) on day 250 (Table 8.1, Appendix 9.1.1). Control and 15 ppm animals survived the entire study period without incident. The authors considered the moribund condition of 14 M to have developed spontaneously, unrelated to atrazine treatment. This animal lost 3 kg and became cachectic early in the second month of treatment, following prior bouts of hypoactivity, bloody discharge from the penis, fecal changes (bloody, mucoid, and/or soft), mydriasis, and reduced pupillary response. Clinical laboratory changes included elevated white cell count, increased serum globulin level, and depressed erythroid parameters. Principal histopathological findings were stated to be consistent with published reports of disseminated fibrinoid necrotic arteritis (termed "polyarteritis nodosa," or, alternatively, necrotizing vasculitis), considered a spontaneous disease in the dog, involving meningeal, coronary, renal, and other blood vessels (mesenteric, testicular, etc.).

Whereas the authors considered the moribund condition of 39 F (1000 ppm) to be related to compound administration, they considered that of 16 M receiving the same compound level unrelated to treatment. 39 F displayed a syndrome of clinical changes including an abnormal EKG profile, ascites and cachexia, referable to cardiopathy. Pathological changes included abundant clear ascites fluid; cardiac dilatation, and liver adhesions. Histologic findings included degenerative lesions (both atrophy

and myelosis) of the atrial myocardium, as well as hepatic centrilobular necrosis. On the other hand, 16 M manifested a persistent and progressive dermatitis with development of sores and scabs, and failure to gain weight associated with low food consumption, but none of the clinical signs referable to cardiac insufficiency. Necropsy findings included skin lesions associated with Demodex sp. mites, but also degenerative lesions in both atrial myocardia. Hence, while the moribundity of 16 M was considered to be due to the debilitating, progressive dermatitis caused by mites, the authors admit the possibility that atrazine could have contributed to the debilitated state, because of the presence of "compound-related myocardial lesions."

Among animals on 1000 ppm atrazine, treatment-related clinical signs attributable to atrazine-induced myocardial degeneration (discussed below) included ascites (one each high-dose male, 15 M, and female 39 F), cachexia (one male, 14 M, 150 ppm; 1 female, 39 F, 1000 ppm) and labored/shallow breathing (15 M, 1000 ppm). Both of these high-dose animals as well as three others fed at 1000 ppm (19 M, 20 M, and 40 F) had EKG and/or morphologic evidence of heart disease (characterized as irregular heartbeat, tachycardia, and increased heart rate), in contrast to the mid-dose dog, 14 M, which did not. These treatment-related clinical signs correlated to pathologic findings attributed to atrazine administration.

A variety of other clinical signs, considered by the authors to be spontaneous findings commonly found in laboratory dogs were recorded (see Report Table 8.1 attached to this review). Since they occurred randomly and were not dose-related, they were considered to be unrelated to atrazine treatment.

Body Weight/Food Consumption

High-dose males and females ate less feed from the first week of treatment, which caused (at least in part) a parallel reduction in body weight or body weight gain (Figures 7.1, 7.2, 7.3, and 7.4; Table 8.4; Appendix 9.1.4). Compared to baseline (pretreatment) values, percent body weight gain at study termination for the 1000 ppm group was +29.7 percent for males and 21.5 percent for females, whereas for controls the gain was 46.6 and 35.6 percent, respectively (Report Table 8.2, attached to this review). However, only the change in males was significant, at $p < 0.05$, using the two-tailed Dunnett's test. Treatment-related, statistically significant reductions in food consumption were found in high-dose males and females during the first quarter of the study period (Table 8.4). Where differences were not significant, food consumption was recorded as consistently less than in controls. No statistically significant reduction in body weight or percent body weight gain and food consumption were recorded in males or females ingesting feed containing 15 or 150 ppm atrazine.

Ophthalmoscopy

No compound-related ocular changes were found on ophthalmoscopic examinations (Appendix 9.2). A summary of individual ophthalmoscopic findings revealed comparable incidences and types of changes in control as in test groups.

Electrocardiography

Treatment-related EKG changes were stated in the text of the report to be found only in the high-dose groups at various time points of recording (Appendix 9.3). Statistically significant findings in males were recorded for increased heart rate on days 85, 175, and 361; decreased P-II (height of P wave) on days 85, 175, 267, and 361; decreased PR-interval time on day 361; and decreased QT-interval time on days 267 and 361. Statistically significant findings in females consisted of decreased P-II on days 85, 175, 267, and 361; decreased PR on day 175; and increased heart rate on day 175.

The authors noted other statistically significant EKG changes in all test groups, but these were considered "incidental and unrelated to treatment." They included decreased mean electrical axis at day 175 and increased P-II and PR at day 267 in low-dose females; increased R waves at day 361 in mid-dose, and at days 267 and 361 in high-dose males; but also significantly decreased P-II of 0.200 mV ($p < 0.05$) in mid-dose (150 ppm) females at day 175. (This is the same change considered treatment-related in the high-dose group, with a mean value of = 0.100 mv at day 175 in high-dose females, $p < 0.01$.) The value in 150 ppm females was stated to be within the normal background range of values for the species. (Background values were not provided in the report, however, but the concurrent control value was given as 0.267 mV.) The staff cardiologist for Ciba-Geigy also noted arrythmia (atrial fibrillation) in four high-dose dogs (two males and two females) at various time points, and atrial premature complexes in one high-dose female at day 361 (Appendix 9.3).

Hematology

Treatment-related changes in hematological values were reported to have occurred in the high-dose group only (Table 8.5 and Appendix 9.1.5). These changes consisted of slight but statistically significant reductions in erythroid parameters (red cell count, hemoglobin, and hematocrit) in males only throughout the study (considered secondary to body weight depression), and mild increases in platelet counts in both sexes (said to be "minimal" by the authors), and of unclear toxicologic significance, since this increase did not correlate to any pathologic observation. Additionally, sporadic statistically significant

alterations in other hematologic parameters, considered by the authors to be toxicologically unimportant, occurred in males of all test groups (MCV, WBC, eosin) and in mid- and high-dose females (lymp mono, WBC, MCHB, and MCHC). All these changes were considered "spontaneous and unrelated to treatment," since neither dose-response nor consistent time-response occurred, they were not associated with any morphologic findings, and group mean values were generally within the range of concurrent control values, as well as historical background for differential white blood cell counts (stated as "MIN" and "MAX" in Table 8.5).

Biochemistry

The only compound-related biochemical changes reported were slight decreases in total protein and albumin, statistically significant ($p < 0.05$) for high-dose males and considered secondary to reduced feed consumption (Table 8.6 and Appendix 9.1.6). Other statistically significant differences occurred sporadically in low- and high-dose males and females, and were considered spontaneous changes unrelated to treatment, since they were neither dose- nor time-related, but marginal and within concurrent control values. Among low-dose animals, findings consisted of increased total bilirubin in males and increased phosphorus in females; in high-dose groups calcium was decreased and chloride increase in males, while females had increased sodium and glucose.

Urinalysis

No stated compound-related changes in urinary values were found, although several sporadic statistically significant differences occurred in all test groups (Table 8.7 and Appendix 9.1.7). Low-, mid-, and high-dose females had nondose-related increased numbers of epithelial cells at day 175 only; in addition, high-dose females had decreased protein at day 270. Decreased crystals and specific gravity were noted in mid-dose males at day 89; on the other hand, high-dose males manifested increased crystals at day 89, decreased epithelial cell number at day 357, and decreases in WBC at day 175. All these changes were considered spontaneous, with no consistency, and within the range of concurrent control values observed in this study.

Organ Weights

Treatment-related, statistically significant changes in organ weights were recorded for high-dose animals, specifically decreases in absolute (but not relative) heart weight in females, and increased relative liver weight (both body and brain weight ratios) in males (Table 8.8 and Appendix 9.1.8). The cardiac changes were considered a direct effect of atrazine administration, while the liver weight change was primarily due to one animal (15 M) and considered secondary to ascites. Other

statistically significant changes in organ weights occurred in mid- and high-dose animals, but these were considered unrelated to treatment. For example, mid-dose females manifested marginal increases in absolute heart weight and heart/brain ratios inconsistent with the treatment-related decreases at the higher dose. High-dose females had increased relative (to body weight) ovary weights, consistent with stages of estrus rather than atrazine administration.

Gross Pathology

Gross pathological changes considered to be related to atrazine administration were found in the majority of high-dose survivors (4/5 males and 5/5 females) and consisted mainly of moderate to severe dilatation of right and/or left atria; less common cardiac changes included fluid-filled pericardium and enlarged heart in three males (Report Tables 3 and 4 of Appendix 9.4, attached to this review). Secondary changes included abdominal ascites and liver adhesions, the most severe noted in two animals (15 M and 39 F), one of which was sacrificed moribund before study termination (39 F).

Microscopic Pathology

Microscopic findings were correlated to the gross changes, cardiac lesions occurring most often and restricted to high-dose males and females (Table 5 of Appendix 9.4, attached). The principal histologic lesion observed was atrophy and myelosis of the atrial myocardium, with atrial edema. Less common lesions in high-dose animals included centrilobular hepatic necrosis (two females) and serous lymphadenitis of the mesenteric lymph node (one male and one female). These treatment-related gross and histologic changes were also correlated to EKG abnormalities.

Summary

In summary, the authors concluded that treatment-related effects of atrazine feeding were found only at the highest dietary level (1000 ppm = 33.65 mg/kg/day for males; 33.80 mg/kg/day for females), and included (as taken directly from the Final Report):

1. At least one death (female) [and possibly two, if one includes 16 M];

2. Cachexia and ascites;

3. Reduction in body weight and percent body weight gain;

4. Reduction in food consumption;

5. Irregular heartbeat and increased heart rate;

6. EKG alterations such as increased heart rate, decreased P-II values, atrial premature complexes, and atrial fibrillation;

7. Slight changes in hematological parameters such as decreased erythroid values and increased platelet counts;

8. Slightly decreased serum (total) protein and albumin;

9. Slightly decreased absolute heart weight in females and slightly increased relative liver weights in males;

10. Moderate to severe cardiac lesions consisting primarily of dilatation of right and/or left atria and myocardial degeneration (atrophy, myelosis) of the atria. The cardiac lesions were considered direct effects of atrazine administration, whereas many of the additional findings (clinical, hepatic, etc.), were considered secondary.

From these results the authors proposed that the NOEL in this dog study was 150 ppm (actual intake, 4.97 mg/kg/day for both sexes), and based upon the cardiotoxicity observed, the MTD was exceeded at 1000 ppm.

A Quality Assurance (QA) Statement was present, attesting to repeat inspections/audits of this study, and signed by the Director of QA/Regulatory Compliance, October 27, 1987.

TB Evaluation: Core-Minimum Data

Doses administered: 0, 15, 150, and 1000 ppm (equivalent to measured intakes of: 0, 0.48, 4.97, and 33.65/33.80 [M/F] mg/kg/day.

The most significant effect of atrazine administration described in this 1-year dog study was the syndrome of cardiopathy, featuring discrete myocardial degeneration and most prominently found in the test group receiving the highest concentration of dietary atrazine, 1000 ppm (equivalent to actual intakes of 33.65 mg/kg/day for males, 33.80 mg/kg/day for females). Clinical signs referable to cardiac toxicity, such as ascites, cachexia, labored/shallow breathing,and abnormal EKG, were first observed as early as 17 weeks into the study. Gross pathological examination revealed moderate to severe dilatation of the right atrium (and occasionally the left atrium), microscopically manifest as atrophy and myelosis (degeneration of the atrial myocardium). The authors proposed that the NOEL was 150 ppm (4.97 mg/kg/day) for both sexes.

While conceding that the MTD was exceeded at 1000 ppm, we

question whether 150 ppm represents a valid no-effect level. Two mid-dose males manifested some cardiac involvement, which was discounted by the authors as not treatment-related. Animal 12 M had a "moderate" degree of dilatation of the right atrium, combined with "minimal" dilatation of the left atrium, plus a "pale lesion of the epicardium of the left ventricle" on gross examination (but it was asserted that no microscopic atrial lesions were found). Animal 14 M was sacrificed moribund during the 11th treatment week manifesting clinical signs such as hypoactivity and cachexia (among other changes). Gross pathological examination of this animal revealed "red" right atrium, histologically manifest as a "thickened" atrium with edema. The cause of death was stated to be consistent with disseminated arteritis, reported to occur spontaneously in beagle dogs, and termed "polyarteritis nodosa." Additional support for considering 150 ppm an effect level is provided by significantly decreased P-II waves in mid-dose females at day 175 of the study.

If these cardiac changes at the mid-dose level represent the lower tier of atrazine effects, then this intermediate dose should be considered an effect level (LEL); thus, the next lowest dose level (15 ppm (0.48 mg/kg/day), becomes the NOEL. This would conform to the TSCA Test Guidelines, which advise that the intermediate dose produce a low level of toxicity, and there be a gradation of effects from the appropriate spacing of doses.

Attachments

ATRAZINE TECHNICAL: 52-WEEK ORAL FEEDING STUDY IN DOGS (MIN 852008)

TABLE 8.1.

Summary incidence of mortality and clinical signs[a]

Sex:	Males				Females			
Group No.:	1	2	3	4	1	2	3	4
Dose (ppm):	0	15	150	1000	0	15	150	1000
No. of Dogs :	6	4	4	6	6	4	4	6
Observations								
Mortality								
Sacrificed moribund	0	0	1	1	0	0	0	1
Abdomen distended	0	0	0	0	0	1	0	0
Abscess w/wo discharge	0	0	0	1	0	0	0	0
Alopecia	1	0	0	0	0	0	0	1
Ascites	0	0	0	1	0	0	0	1
Blood from penis	0	0	1	0	0	0	0	0
Cachexia	0	0	1	0	0	0	0	1
Dermatitis	1	1	1	1	2	0	2	2
with scabs	0	0	0	1	0	0	0	1
with sore	0	0	0	1	0	0	0	1
Emesis	1	2	2	3	0	3	1	3
with food	1	0	1	0	0	0	0	0
Feces: blood	4	0	2	3	3	2	0	2
diarrhea	3	0	1	0	1	2	2	2
mucoid	3	3	3	6	5	3	3	4
soft	3	2	2	2	1	3	1	2
Hypoactivity	2	0	1	2	0	0	0	1
Labored breathing	0	0	0	1	0	0	0	0
Limp	0	0	0	1	0	0	0	0
Miosis	1	0	0	0	0	0	0	0
Mydriasis	0	1	2	0	0	0	1	1
Red discharge - location unknown	0	0	0	1	0	0	0	0
Red discharge - vaginal	0	0	0	0	1	0	0	0
Reduced pupillary response	0	2	3	1	0	0	2	1
Ptosis	0	0	0	0	0	0	0	1
Salivation	1	0	0	0	0	0	0	0
Scab	0	0	0	1	0	0	0	0
Shallow breathing	0	0	0	1	0	0	0	0
Sore	1	0	0	0	2	1	1	1
Swollen appendage	1	0	0	0	1	0	1	0

[a]The incidence reported is based on the number of animals displaying the clinical sign on at least one occasion during the study.

ATRAZINE TECHNICAL: 52-WEEK ORAL FEEDING STUDY IN DOGS (MIN 852008)

TABLE 8.2.

Mean body weight change during the 52-week dosing period[a]

Group	Dose (ppm)	Males			Females		
		Body Weight (kg)		Percent[b]	Body Weight (kg)		Percent[b]
		Baseline	Week 52	Change	Baseline	Week 52	Change
1	0	8.0	11.7	+46.6	7.3	9.9	+35.6
2	15	7.6	11.6	+52.5	6.8	9.9	+44.8
3	150	8.4	11.5	+36.8	7.0	9.5	+35.9
4	1000	7.2	9.4	+29.7[c]	6.9	8.3	+21.5

[a]These data are taken directly from the computer printout.

[b]Relative to the baseline body weight.

[c]$p < 0.05$ using a two tailed Dunnett T test.

ATRAZINE TECHNICAL: 52-WEEK ORAL FEEDING STUDY IN DOGS (MIN 852008)
PATHOLOGY REPORT

Table 3

Gross cardiac changes observed in dogs euthanized at the end of the dosing period

Male Number		Female Number	
Group 1 (0 ppm)			
1	None	21	Right atrium, minimal dilatation
2	Right atrium, minimal dilatation	22	None
3	None	23	Right atrium, minimal dilatation
4	None	24	None
5	Left atrium, minimal dilatation	25	None
6	Right atrium, minimal dilatation	26	None
Group 2 (15 ppm)			
7	None	27	None
8	None	28	None
9	None	29	Right atrium, minimal dilatation
10	None	30	None
Group 3 (150 ppm)			
11	None	31	Atria (2), minimal dilatation
12	Right atrium, moderate dilatation Left atrium, minimal dilatation Left ventricle, epicardium pale lesion	32	None
		33	None
		34	Right atrium, minimal dilatation
13	None		
14	(Early Death)		
Group 4 (1000 ppm)			
15	Heart, enlarged and soft; pericardium, fluid; right atrium, moderate dilatation; left atrium, minimal dilatation; left ventricle, pale lesion	35	Right atrium, moderate dilatation Left atrium, minimal dilatation

(Continued)

ATRAZINE TECHNICAL: 52-WEEK ORAL FEEDING STUDY IN DOGS (MIN 852008)
PATHOLOGY REPORT

Table 3 (cont.)

Gross cardiac changes observed in dogs euthanized at the end of the dosing period

Male Number		Female Number	
Group 4 (1000 ppm) (cont.)			
16	(Early Death)	36	Right atrium, moderate dilatation; left atrium, minimal dilatation
17	Right atrium, severe dilatation; left atrium, minimal dilatation	37	Atria (2), severe dilatation
18	Right atrium, moderate dilatation; left atrium, minimal dilatation	38	Right atrium, moderate dilatation; left atrium, minimal dilatation
19	Heart, enlarged and soft; atria (2), severe dilatation	39	(Early Death)
20	None	40	Right atrium, minimal dilatation; left atrium, moderate dilatation

ATRAZINE TECHNICAL: 52-WEEK ORAL FEEDING STUDY IN DOGS (MIN 852008)
PATHOLOGY REPORT

Table 4

Summary of gross findings

SEX:	MALES				FEMALES			
GROUP:	1	2	3	4	1	2	3	4
NUMBER OF ANIMALS EXAMINED:	6	4	4	6	6	4	4	6
ORGAN OR SITE								
HEART								
Dilatation								1
Enlarged				2				
Texture, soft				2				
HEART - LEFT ATRIUM								
Dilatation	1		1	4			1	5
HEART - LEFT VENTRICLE EPICARDIUM								
Lesion, pale			1					
HEART - LEFT VENTRICLE MYOCARDIUM								
Lesion, pale				1				
HEART - PERICARDIUM								
Contents, fluid substance				1				
HEART - RIGHT ATRIUM								
Dilatation	2		1	4	2	1	2	5
Color, red			1					
HEART - RIGHT ATRIUM EPICARDIUM								
Contents, fluid substance			1					
KIDNEY								
Mottled			1					
LIVER								
Adhesions								1
Color, darker than normal				1				
Enlarged				1				
Texture, firm				1				
LUNGS								
Color, pallid		1						
Lesion, dark				1				
Lesion, red		1		1				
Lesion, tan								1
Lesion, white		1						1

(Continued)

ATRAZINE TECHNICAL: 52-WEEK ORAL FEEDING STUDY IN DOGS (MIN 852008)
PATHOLOGY REPORT

Table 4 (cont.)

Summary of gross findings

SEX:	MALES				FEMALES			
GROUP:	1	2	3	4	1	2	3	4
NUMBER OF ANIMALS EXAMINED:	6	4	4	6	6	4	4	6
ORGAN OR SITE								
LYMPH NODE - AXILLARY								
Enlarged			1					
LYMPH NODE - MEDIASTINAL								
Color, red			1					
Enlarged			1					
LYMPH NODE - MESENTERIC								
Color, red				1				1
Enlarged				1				1
Texture, soft								1
PERITONEAL CAVITY - GENERAL								
Contents, clear substance								1
Contents, fluid substance				1				1
PITUITARY								
Cystic								1
PREPUCE - EPIDERMIS								
Lesion				1				
PROSTATE								
Smaller than normal				1				
SKELETAL MUSCLE - HEAD								
Lesion, white			1					
SKIN								
Lesion, red	1							
SKIN - ABDOMEN								
Lesion, red						1		
SKIN - NECK								
Lesion				1				
Lesion, red								1
SKIN - POSTAPPENDAGE								
Lesion				1				
Left swollen				1				

(Continued)

ATRAZINE TECHNICAL: 52-WEEK ORAL FEEDING STUDY IN DOGS (MIN 852008)
PATHOLOGY REPORT

Table 4 (cont.)

Summary of gross findings

SEX:	MALES				FEMALES			
GROUP:	1	2	3	4	1	2	3	4
NUMBER OF ANIMALS EXAMINED:	6	4	4	6	6	4	4	6
ORGAN OR SITE								
SKIN - SHOULDERS								
Lesion				1				
SPLEEN								
Smaller than normal				1				
TESTIS								
Dark lesions, bilateral			1					
THYMUS								
Contents, fluid substance			1					
URINARY BLADDER								
Calculi					1			
Color, red					1			
Thickened					1			

ATRAZINE TECHNICAL: 52-WEEK ORAL FEEDING STUDY IN DOGS (MIN 852008)
PATHOLOGY REPORT

Table 5

Summary of microscopic observations

SEX:	MALES				FEMALES			
GROUP:	1	2	3	4	1	2	3	4
DOSE LEVEL (PPM):	0	15	150	1000	0	15	150	1000
ORGAN OR SITE (*)								
ADRENAL	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)
AORTA	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)
BONE - PHALANGES POSTERIOR LEFT	(0)	(0)	(0)	(1)	(0)	(0)	(0)	(0)
Proliferation				1				
BONE MARROW	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)
BRAIN	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)
CECUM	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)
COLON	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)
DUODENUM	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)
EPIDIDYMIS	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)
Focal Acute Purulent Inflammation			1					
ESOPHAGUS	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)
EYE	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)
GALLBLADDER	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)
Edema	1			1				1
HEART - CORONARY ARTERY	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)
Necrosis			1					
Periarteritis Nodosa			1					
Focal Subacute Lymphocytic Inflammation			1					
HEART - LEFT ATRIUM MYOCARDIUM	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)
Edema								1
Focal Edema								1
Fatty Infiltration				1	1			
Focal Atrophy				2				5***
Myolysis				3				6***

(Continued)

*=(Number of Tissues Examined)
**=Statistically Significant at $p \leq .05$
***=Statistically Significant at $p \leq .01$
G7/2 (MIN 852008)

ATRAZINE TECHNICAL: 52-WEEK ORAL FEEDING STUDY IN DOGS (MIN 852008)
PATHOLOGY REPORT

Table 5 (cont.)

Summary of microscopic observations

SEX:	MALES				FEMALES			
GROUP:	1	2	3	4	1	2	3	4
DOSE LEVEL (PPM):	0	15	150	1000	0	15	150	1000
ORGAN OR SITE (*)								
HEART - MYOCARDIUM	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)
Necrosis			1					
Focal Subacute Lymphocytic Inflammation			1					
HEART - PAPILLARY MUSCLE	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)
Myolysis				1				
HEART - RIGHT ATRIUM EPICARDIUM	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)
Edema			1					
Thickened			1					
HEART - RIGHT ATRIUM MYOCARDIUM	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)
Edema				1				1
Focal Edema								1
Focal Atrophy				5***				5***
Myolysis				4**				4**
HEART-RIGHT VENTRICLE ARTERIES	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)
Perivascular Cuffing			1					
ILEUM	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)
JEJUNUM	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)
KIDNEY - PAPILLAE DUCTS	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)
Focal Calcification		1						
LIVER	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)
Focal Subacute Lymphocytic Inflammation				1				
Telangiectasis				1				
Fibrosis				1				
LIVER - LOBULE CENTRO	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)
Focal Hemorrhage								1
Focal Necrosis								1

(Continued)

*=(Number of Tissues Examined)
**=Statistically Significant at $p \leq .05$
***=Statistically Significant at $p \leq .01$

ATRAZINE TECHNICAL: 52-WEEK ORAL FEEDING STUDY IN DOGS (MIN 852008)
PATHOLOGY REPORT

Table 5 (cont.)

Summary of microscopic observations

SEX:	MALES				FEMALES			
GROUP:	1	2	3	4	1	2	3	4
DOSE LEVEL (PPM):	0	15	150	1000	0	15	150	1000
ORGAN OR SITE (*)								
LIVER - SEROSA	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)
Fibrosis								1
LUNGS	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)
Acute Purulent Inflammation				1				
Bronchopneumonia	1			1	1			
Focal Congestion		1						
Focal Hemorrhage						1		
Focal Subacute Lymphocytic Inflammation	1							
Foreign Body	1							
LUNGS - ALVEOLAR SEPTA	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)
Focal Acute Purulent Inflammation				1				
Fibrosis				1				
LUNGS - ALVEOLI	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)
Focal Acute Purulent Inflammation				1				
Hemosiderosis				1				
LUNGS - CAPSULE	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)
Fibrosis		1						
Focal Subacute Lymphocytic Inflammation		1						
LYMPH NODE - AXILLARY	(6)	(3)	(3)	(5)	(6)	(4)	(4)	(6)
LYMPH NODE - MEDIASTINAL	(0)	(0)	(1)	(0)	(0)	(0)	(0)	(0)
Serous Inflammation			1					
LYMPH NODE - MESENTERIC	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)
Congestion				1				
Serous Inflammation				1				1
MAMMARY GLAND (see Skin-Mammae)								

(Continued)

*=(Number of Tissues Examined)
**=Statistically Significant at $p \leq .05$
***=Statistically Significant at $p \leq .01$

ATRAZINE TECHNICAL: 52-WEEK ORAL FEEDING STUDY IN DOGS (MIN 852008)
PATHOLOGY REPORT

Table 5 (cont.)

Summary of microscopic observations

SEX:	MALES				FEMALES			
GROUP:	1	2	3	4	1	2	3	4
DOSE LEVEL (PPM):	0	15	150	1000	0	15	150	1000
ORGAN OR SITE (*)								
MESENTERY - ARTERIES	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)
Medial Fibrinoid Degeneration			1					
Necrosis			1					
Periarteritis Nodosa			1					
Focal Subacute Lymphocytic Inflammation			1					
Thrombosis			1					
OPTIC NERVE	(6)	(4)	(4)	(6)	(5)	(4)	(4)	(6)
OVARY					(6)	(4)	(4)	(6)
Immature								1
PANCREAS	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)
PARATHYROID	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)
Cystic	1				1		1	
PITUITARY	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)
Cystic				1	1		1	2
PREPUCE - EPIDERMIS	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)
Focal Chronic Lymphocytic Inflammation				1				
PROSTATE	(6)	(4)	(4)	(6)				
RECTUM	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)
SALIVARY GLAND	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)
SCIATIC NERVE	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)
SKELETAL MUSCLE - THIGH	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)
SKELETAL MUSCLE - HEAD	(0)	(0)	(1)	(0)	(0)	(0)	(0)	(0)
Subacute Hyperplastic Inflammation			1					

(Continued)

*=(Number of Tissues Examined)
**=Statistically Significant $p \leq .05$
***=Statistically Significant $p \leq .01$

ATRAZINE TECHNICAL: 52-WEEK ORAL FEEDING STUDY IN DOGS (MIN 852008)
PATHOLOGY REPORT

Table 5 (cont.)

Summary of microscopic observations

SEX:	MALES				FEMALES			
GROUP:	1	2	3	4	1	2	3	4
DOSE LEVEL (PPM):	0	15	150	1000	0	15	150	1000
ORGAN OR SITE (*)								
SKIN - DERMIS ABDOMEN	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)
Focal Subacute Lymphocytic Inflammation					1			
SKIN - EPIDERMIS SHOULDERS	(0)	(0)	(0)	(1)	(0)	(0)	(0)	(0)
Hyperplasia				1				
Parakeratosis				1				
Demodex Folliculorum				1				
SKIN - DERMIS SHOULDERS	(0)	(0)	(0)	(1)	(0)	(0)	(0)	(0)
Focal Subacute Lymphocytic Inflammation				1				
SKIN - MAMMAE					(6)	(4)	(4)	(6)
Functional Hypertrophy					1	1	2	1
SKIN - NECK	(0)	(0)	(0)	(1)	(0)	(0)	(0)	(0)
Demodex Folliculorum				1				
SKIN - POSTAPPEND LEFT	(0)	(0)	(0)	(1)	(0)	(0)	(0)	(0)
Acute Purulent Inflammation				1				
Ulcer				1				
SPINAL CORD	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)
SPLEEN	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)
Constriction				1				
Hemosiderosis								1
SPLEEN - RED PULP	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)
Hemosiderosis				2				2
STERNUM	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)
STOMACH	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)

(Continued)

*=(Number of Tissues Examined)
**=Statistically Significant at $p \leq .05$
***=Statistically Significant at $p \leq .01$

ATRAZINE TECHNICAL: 52-WEEK ORAL FEEDING STUDY IN DOGS (MIN 852008)
PATHOLOGY REPORT

Table 5 (cont.)

Summary of microscopic observations

SEX:	MALES				FEMALES			
GROUP:	1	2	3	4	1	2	3	4
DOSE LEVEL (PPM):	0	15	150	1000	0	15	150	1000
ORGAN OR SITE (*)								
TESTIS	(6)	(4)	(4)	(6)				
Hypospermatogenesis				1				
Focal Hemorrhage			1					
Focal Acute Purulent Inflammation			1					
Focal Coagulative Necrosis			1					
TONGUE	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)
THYMUS	(6)	(4)	(3)	(6)	(6)	(4)	(4)	(6)
Atrophy				1				
Cystic		1		1		1		
THYMUS - ARTERIES	(6)	(4)	(3)	(6)	(6)	(4)	(4)	(6)
Fibrinoid Degeneration			1					
Periarteritis Nodosa			1					
THYROID	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)
Anomaly							1	
THYROID - INTERSTITIAL CELLS	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)
Hyperplasia								1
TRACHEA - EPITHELIUM	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)
Focal Acute Mucous Inflammation		1						
URINARY BLADDER - EPITHELIUM	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)
Hyperplasia					1			
URINARY BLADDER - NECK	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)
Ulcer				1				
URINARY BLADDER - SUBMUCOSA	(6)	(4)	(4)	(6)	(6)	(4)	(4)	(6)
Chronic Lymphocytic Inflammation					1			
Edema					1			

(Continued)

*=(Number of Tissues Examined)
**=Statistically Significant at $p \leq .05$
***=Statistically Significant at $p \leq .01$

ATRAZINE TECHNICAL: 52-WEEK ORAL FEEDING STUDY IN DOGS (MIN 852008)
PATHOLOGY REPORT

Table 5 (cont.)

Summary of microscopic observations

SEX:	MALES				FEMALES			
GROUP:	1	2	3	4	1	2	3	4
DOSE LEVEL (PPM):	0	15	150	1000	0	15	150	1000
ORGAN OR SITE (*)								
UTERUS					(6)	(4)	(4)	(6)
UTERUS - HORN					(6)	(4)	(4)	(6)
Normal Pregnancy								1
VAGINA					(6)	(4)	(4)	(6)

*=(NUmber of Tissues Examined)
**=Statistically Significant at $p \leq .05$
***=Statistically Significant at $p \leq .01$

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

006718

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

Mar 24,1988

MEMORANDOM

SUBJECT: Atrazine, Review of Dermal Absorption Studies

TO: Judith Hauswirth Ph.D
Section Head
Review Secion VI

FROM: Robert P. Zendzian PhD
Senior Pharmacologist
Toxicology Branch
HED (TS-769)

Compound; Atrazine — Tox Chem #63

Registration #100-529 — Registrant; Ciba-Geigy

Accession #404313-08&-11 — Tox Project #8-0320A

Action Requested

Review the following studies

Dermal absorption of ^{14}C-Atrazine by rats (general metabolism), G.J. Marco, Biochemistry Dept., Agricultural Division, Ciba-Geigy Corp. Study No. ABR-83005; 5/16/83, MIRD 404313-11.

Dermal absorption of ^{14}C-Atrazine by rats (general metabolism), T. Murphy, Biochemistry Dept., Agricultural Division, Ciba-Geigy Corp. Study No. ABR-87098; 11/6/87, MIRD 404313-08.

This document contains the following report which describes the in life portion of the study;

Dermal absorption of ^{14}C-Atrazine in Rats, E.M. Craine, WIL Research Laboratories, Project No. WIL-82015, 11/5/87.

Conclusions

Study No. MIRD 404313-11

Core Classification Unacceptable

In general the report was so poorly written as to make it impossible to determine the experimental design while the

Data Evaluation Report 006718

Compound Atrazine

Citation

Dermal absorption of ^{14}C-Atrazine by rats (general metabolism), G.J. Marco, Biochemistry Dept., Agricultural Division, Ciba-Geigy Corp. Study No. ABR-83005; 5/16/83, MIRD 404313-11.

3/5-4/88

Reviewed by Robert P. Zendzian Ph.D.
Senior Pharmacologist

Core Classification Unacceptable

Conclusions

In general the report was so poorly written as to make it impossible to determine the experimental design while the methodology lacked sufficient detail to allow evaluation. However, deficiencies were identified that are sufficient to invalidate the study. These include the following;

1. Compound was applied in ethanol, not in the field solvent. Since the dermal absorption of a compound is dependent upon the solvent, use of the wrong solvent will produce unusable data.

2. The application site was not covered allowing material to flake off. This would both decrease the amount of material available for absorption and contaminate the urine and feces.

methodology lacked sufficient detail to allow evaluation. However, deficiencies were identified that are sufficient to invalidate the study. These include the following;

1. Compound was applied in ethanol, not in the field solvent. Since the dermal absorption of a compound is dependent upon the solvent, use of the wrong solvent will produce unusable data.

2. The application site was not covered allowing material to flake off. This would both decrease the amount of material available for absorption and contaminate the urine and feces.

Study No. MRID 404313-08

Core Classification Acceptable

Atrazine in 4L formulation is absorbed in relatively small amounts through the skin. Typical values are 2.00, 0.53 and 0.26 % for 10 hour exposures to doses of 0.01, 0.1 or 1.0 mg/cm^2. Significant quantities remain on the skin after washing with soap and water (24.87, 21.10 and 10.49 %). No significant differences in absorption were observed between the 4L and 80W formulations tested at 1.0 mg/cm^2 for 10 hours. The data indicate that absorption is approaching saturation at the high dose.

Attachments

DERs
One-Liner

Data Evaluation Report 006718

Compound Atrazine

Citation

Dermal absorption of ^{14}C-Atrazine by rats (general metabolism), T. Murphy, Biochemistry Dept., Agricultural Division, Ciba-Geigy Corp. Study No. ABR-87098; 11/6/87, MIRD 404313-08.

This document contains the following report which describes the in life portion of the study;

Dermal absorption of ^{14}C-Atrazine in Rats, E.M. Craine, WIL Research Laboratories, Project No. WIL-82015, 11/5/87.

Reviewed by Robert P. Zendzian Ph.D. 7/5/88
Senior Pharmacologist

Core Classification Acceptable

Conclusions

Atrazine in 4L formulation is absorbed in relatively small amounts through the skin. Typical values are 2.00, 0.53 and 0.26 % for 10 hour exposures to doses of 0.01, 0.1 or 1.0 mg/cm^2. Significant quantities remain on the skin after washing with soap and water (24.87, 21.10 and 10.49 %). No significant differences in absorption were observed between the 4L and 80W formulations tested at 1.0 mg/cm^2 for 10 hours. The data indicate that absorption is approaching saturation at the high dose.

Materials

Artazine uniformly ring labeled,

low and mid doses
22.0 uCi/mg, 99.5%

high doses
2.3 uCi/mg, 99.0%

Crl:CD®BR male rats 27-41 days old from Charles River Breeding laboratories

Experimental design and methods

Dose preparation and sample analysis was performed at Ciba-Geigy and the in life portion of the study at WIL.

"The low dose was prepared by mixing throughly 4.0 mg of ^{14}C-Atrazine and 5.3 mg of the formulant (4L), then suspending the mixture in 2.0 ml of deionized water. The middose was

prepared by mixing 40 mg of ^{14}C-Atrazine and 53.0 mg of blank formulation (4L) and then suspending the mixture in 2.0 ml of deionized water."

"The 4L high dose formulation was prepared by mixing throughly 530 mg of formulant and 400.0 mg of ^{14}C-Atrazine, then suspending the mixture in 4.0 ml of water. The 80W high dose was prepared by mixing 200.0 mg of ^{14}C-Atrazine and 50.0 mg blank formulant, then suspending the mixture in 2.0 ml of deionized water.

Two groups of 16 and one group of 20 male rats were treated dermally with single doses of ^{14}C-atrazine at 0.1, 1.0 and 10.0 mg/rat (0.01, 0.1 and 1.0 mg/cm^2) respectively. Four animals at each dose were dosed with 4L formulation and exposed for 2, 4, 10 and 24 hours. The remaining four animals at 10.0 mg/rat were dosed with 80W formulation and exposed for 10 hours.

"The test material preparations were stored frozen, warmed to room temperature and sonicated 10 minutes prior to analysis and dosing on the appropriate test material application day."

The anterior dorsal hair was shaved from each rat and the area washed with acetone 24 hours prior to dosing. Test material was applied to a 2.5 x 4 cm (10cm^2) area by pipette. The application site was covered with a protective device consisting of a stomahesive bandage as a wall and a filter paper cover.

Animals were individually caged in metabolism cages and total urine and feces collected.

Animals were sacrificed at the end of the exposure period. The protective device was removed and washed. The application site was washed with a detergent solution and water rinsed.

Blood, application site skin, skin under the bandage and the carcass were collected.

The following samples from each animal were sent to Ciba-Geigy for analysis;

"pipet washes, urine, feces, washes, extracts, samples from the protective coverings, gauze, blood, skin samples and carcasses,"

<u>Results</u>

Sample analysis for radioactivity at WIL indicated that dosing suspensions were homogenous and of the expected activity.

72

No compound-related effects on the rats were reported.

006718

Dermal absorption data is summarized in Table 1 below and presented in detail in Tables III - VI of the report.

Table 1. Summary of dermal absorption data. All values are means of 4 animals. All animals dosed with 4L formulation except as noted. Data from Tables III - VI of the report.

Dose	Exposure	Absorbed[a]			On skin[b]	Unabsorbed[c]
(mg/cm^2)	(hours)	(%)	(%/hr)	($mgx10^{-5}$)	(%)	(%)
0.01*	2	0.68	0.34	6	23.53	77.25
0.009†	4	1.24	0.31	11	20.56	71.88
	10	2.00	0.20	18	24.87	69.51
	24	4.93	0.21	44	20.72	69.02
0.1	2	0.21	0.11	20	25.06	71.55
0.095	4	0.36	0.09	34	18.97	75.72
	10	0.53	0.05	50	21.10	78.93
	24	1.26	0.05	119	29.04	67.43
1.0	2	0.13	0.06	107	11.24	88.67
0.82	4	0.09	0.02	74	14.69	88.00
	10	0.26	0.03	213	10.49	89.29
	24	0.21	0.01	172	9.58	91.03
1.0 80W	10	0.24	0.02	244	8.81	89.15
1.02						

* Nominal dose.
† Applied dose.
a. Total of blood, carcass, urine and feces.
b. Total of skin I and skin II.
c. Total of bandage rinse, bridge rinse, paper rinse, soap rinse, water rinse, gauze A, gauze B and cage wash.

Discussion

The percent of dose absorbed followed the most common pattern of absorption with the percent increasing with time and decreasing with increasing dose. Significant quantities of test material remained on/in the skin following soap and water wash. There are clear indications that the process is approaching saturation at the high dose in that;

1. The percent absorbed per hour decreased with time in each dose and the proportionate decrease was larger with increasing dose.

2. As the dose increased the total quantities absorbed increased proportionately less per dose increase.

3. The quantity on/in the skin increased ten fold from 0.01 to 0.1 mg/cm^2 but only five fold from 0.1 to 1.0 mg/cm^2.

For regulatory purposes the test material which remains on/in the skin after soap and water wash is considered absorbable. For risk assesments the percent absorbed is added to the percent on/in the skin to determining quantity absorbed. However, the possibility exists that the relatively large quantity remaining on/in the skin is an artifact of the experimental procedure. A recent study, designed to determine if the material remaining on/in the skin after washing could be absorbed, showed that 2 to 3 times more material could be washed from the skin of living animals then from the skin of recently sacrificed animals. In this study the animals were sacrificied before washing the application site.

This possibility may be tested by treating 4 animals per dose for 10 hours exactly as was done in this study but washing the appliction site before sacrificing the animals. The ten hour exposure time is suggested as modeling a worker who washes at the end of the working day.

TABLE III: THE PERCENT OF DOSE ABSORBED[1], UNABSORBED[2], AND REMAINING ON THE SKIN AFTER A SOAP AND WATER RINSE IN ANIMALS TREATED WITH ^{14}C-ATRAZINE AT THE LOW DOSE LEVEL[3]

Fraction	Low Dose (0.1 mg/Rat) Time of Sacrifice (Hours)			
	2	4	10	24
Blood	0.11	0.08	0.10	0.14
Carcass	0.51	1.04	1.37	1.93
Urine	0.06	0.12	0.53	2.53
Feces	0.00	0.00	0.00	0.33
	0.68	1.24	2.00	4.93
Skin I	20.53	18.14	22.33	18.38
Skin II	3.00	2.42	2.54	2.34
Σ Skin	23.53	20.56	24.87	20.72
Absorbed[1]	24.21	21.80	26.87	25.65
Bandage Rinse	0.04	0.07	0.08	0.21
Bridge Rinse	0.16	0.01	0.03	0.03
Paper Rinse	0.07	0.27	0.23	0.55
Soap Rinse	69.46	63.99	61.40	59.74
Water Rinse	5.34	5.78	5.69	6.05
Paper	0.01	0.01	0.02	0.01
Gauze A	1.96	1.61	1.80	1.79
Gauze B	0.07	0.07	0.09	0.10
Cage Wash	0.14	0.07	0.17	0.54
Unabsorbed[2]	77.25	71.88	69.51	69.02
Total ^{14}C Recovered	101.46	93.68	96.38	94.67

[1]Sum of the blood, carcass, urine, feces, skin I, and skin II.

[2]Sum of the bandage rinse, bridge rinse, paper rinse, soap rinse, water rinse, paper, gauze A, gauze B, and cage wash.

[3]Mean of four animals per time point.

TABLE IV: THE PERCENT OF DOSE ABSORBED[1], UNABSORBED[2], AND REMAINING ON THE SKIN AFTER A SOAP AND WATER RINSE IN ANIMALS TREATED WITH ^{14}C-ATRAZINE AT THE MIDDOSE LEVEL[3]

Fraction	Mid Dose (1.0 mg/Rat) Time of Sacrifice (Hours)			
	2	4	10	24
Blood	0.01	0.01	0.01	0.03
Carcass	0.18	0.29	0.38	0.60
Urine	0.02	0.06	0.14	0.58
Feces	0.00	0.00	0.00	0.05
	0.21	0.36	0.53	1.26
Skin I	20.71	15.27	15.39	26.75
Skin II	4.35	3.70	5.71	2.29
Σ Skin	25.06	18.97	21.10	29.04
Absorbed[1]	25.27	19.33	21.63	30.30
Bandage Rinse	0.38	1.05	0.05	1.21
Bridge Rinse	0.01	0.25	0.01	0.01
Paper Rinse	0.02	0.02	0.05	0.10
Soap Rinse	61.53	66.66	70.96	57.28
Water Rinse	6.87	5.27	5.23	7.37
Paper	0.00	0.00	0.00	0.01
Gauze A	2.59	2.36	2.50	1.27
Gauze B	0.14	0.10	0.10	0.09
Cage Wash	0.01	0.01	0.03	0.09
Unabsorbed[2]	71.55	75.72	78.93	67.43
Total ^{14}C Recovered	96.82	95.05	100.56	97.73

[1]Sum of the blood, carcass, urine, feces, skin I, and skin II.

[2]Sum of the bandage rinse, bridge rinse, paper rinse, soap rinse, water rinse, paper, gauze A, gauze B, and cage wash.

[3]Mean of four animals per time point.

TABLE V: THE PERCENT OF DOSE ABSORBED[1], UNABSORBED[2], AND REMAINING ON THE SKIN AFTER A SOAP AND WATER RINSE IN ANIMALS TREATED WITH ^{14}C-ATRAZINE AT THE HIGH DOSE LEVEL[3]

Fraction	High Dose (10.0 mg/Rat) Time of Sacrifice (Hours)			
	2	4	10	24
Blood	0.01	0.00	0.00	0.00
Carcass	0.12	0.08	0.24	0.13
Urine	0.00	0.01	0.02	0.07
Feces	0.00	0.00	0.00	0.01
	0.13	0.09	0.26	0.21
Skin I	7.09	8.80	6.94	6.58
Skin II	4.15	5.89	3.55	3.00
I Skin	11.24	14.69	10.49	9.58
Absorbed[1]	11.37	14.78	10.75	9.79
Bandage Rinse	5.60	4.14	5.75	5.23
Bridge Rinse	0.02	0.02	0.05	0.00
Paper Rinse	0.01	0.01	0.02	0.02
Soap Rinse	76.42	76.98	77.92	77.36
Water Rinse	4.19	3.97	3.11	5.16
Paper	0.00	0.00	0.00	0.00
Gauze A	2.34	2.80	2.35	3.16
Gauze B	0.08	0.07	0.04	0.08
Cage Wash	0.01	0.01	0.05	0.02
Unabsorbed[2]	88.67	88.00	89.29	91.03
Total ^{14}C Recovered	100.04	102.78	100.04	100.82

[1]Sum of the blood, carcass, urine, feces, skin I, and skin II.

[2]Sum of the bandage rinse, bridge rinse, paper rinse, soap rinse, water rinse, paper, gauze A, gauze B, and cage wash.

[3]Mean of four animals per time point.

TABLE VI: COMPARATIVE DATA OF TWO DIFFERENT FORMULATIONS (4L VERSUS 80W) WITH ^{14}C-ATRAZINE TEN HOURS AFTER THE HIGH DOSE LEVEL[1,2,3]

Fraction	High Dose (10.0 mg/Rat) Formulation	
	4L	80W
Blood	0.00	0.00
Carcass	0.24	0.22
Urine	0.02	0.02
Feces	0.00	0.00
	0.26	0.24
Skin I	6.94	4.61
Skin II	3.55	4.20
I Skin	10.49	8.81
Absorbed[1]	10.75	9.05
Bandage Rinse	5.75	0.51
Bridge Rinse	0.05	0.02
Paper Rinse	0.02	0.01
Soap Rinse	77.92	81.22
Water Rinse	3.11	4.25
Paper	0.00	0.00
Gauze A	2.35	3.03
Gauze B	0.04	0.06
Cage Wash	0.05	0.05
Unabsorbed[2]	89.29	89.15
Total ^{14}C Recovered	100.04	98.20

[1]Sum of the blood, carcass, urine, feces, skin I, and skin II.

[2]Sum of the bandage rinse, bridge rinse, paper rinse, soap rinse, water rinse, paper, gauze A, gauze B, and cage wash.

[3]Mean of four animals per time point.

006718

2Reviewed by: Sanford W. Bigelow, Ph.D. 5/6/88
Section VI, Toxicology Branch (TS-769C)
Secondary reviewer: Judith W. Hauswirth, Ph.D. Judith W. Hauswirth 5/9/88
Section VI, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

I. **SUMMARY:**

STUDY TYPE: Metabolism - rat (85-1) **CASWELL NO:** 63

ACCESSION NUMBER: **MRID NO.:** 404313-05

TEST MATERIAL: Atrazine

SYNONYMS: 2-Chloro-4-ethylamino-6-isopropylamino-s-triazine

STUDY NUMBER: ABR-87087

SPONSOR: CIBA-GEIGY Corp., Agricultural Division, P.O. Box 18300 Greensboro, NC 27419 Thomas Parshley, Regulatory Specialist (919) 292-7100 X7207

TESTING FACILITY: CIBA-GEIGY Corp., Biochemistry Dept., P.O. Box 18300 Greensboro, NC 27419
-and-
SRI International, 333 Ravenswood Ave., Menlo Park, CA 94025 (Study No. LSC-1469)
-and-
Agrisearch Incorporated, 26 Water Street, Frederick, MD 21701 (Project No. 1271)

TITLE OF REPORT: Study of delta-^{14}C-Atrazine Dose/Response Relationship in the Rat (General Metabolism).

AUTHOR: B. Thede

REPORT ISSUED: October 23, 1987

CONCLUSIONS:

The distribution of atrazine in rats was found to be dose-dependent. Of the tissues studied, the red blood cells store the highest levels of atrazine, apparently through the covalent binding of a metabolite. In rats exposed to a dose of 100 mg/kg atrazine for 10 days, in decreasing order, the levels found in the following tissues were: red blood cell, liver, kidney, ovary, pituitary, brain, pectoral region of the mammaries. Under the exposure regimen used in this study, atrazine does not accumulate in the tissues of the rat, except perhaps for the red

blood cell. The pattern of atrazine tissue distribution found in this report was similar that found in male rats exposed to a similar dosage regimen (MRID No. 404313-09, Study No. ABR-85104).

The distribution of atrazine was reported to follow first-order kinetics. Two major relationships are found: (1) the whole body half-life, or $t_{1/2}$, and the volume of distribution, or V_d, are independent of the dose of atrazine and (2) the plasma concentration of atrazine and/or its metabolites are directly proportional to the dose of atrazine. Plasma concentrations of atrazine measured during and after atrazine exposure showed that the whole body half-life ($t_{1/2}$) of atrazine or its metabolites is 38.6 hours (1.61 days) in rats. These reported findings further indicate that atrazine does not accumulate under this exposure regimen in the rat.

Summary. The whole body half-life for atrazine is 1.61 days. The red blood cells store the highest concentration of atrazine in the rat, apparently through the covalent binding of a metabolite. Under the dose regimen employed in this study, atrazine does not accumulate in the rat, except perhaps for the red blood cell.

Classification: **Acceptable:** This classification is based on the fact that the methodology requirements established in the Pesticide Assessment Guidelines, Subdivision F §85-1 have been satisfied only for reporting the disposition of atrazine in female rats. However, all of the data requirements for metabolism studies set forth in §85-1 have not been reported.

II. MATERIALS:

A. Test Compound: Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine)

Description: Not provided in this summary report.
Batch #: S85-0653-3
Purity: 98.8% (expiration date - November, 1990)
Radiolabeling procedure:
All carbons in the triazine moiety of atrazine were replaced with carbon-14. The specific activity of the radiolabeled compound (reference CL-IX-77) was 95.8 microCuries/mg in low dose experiments and 1.06 microCuries/mg in the high dose experiments. The purity of the radiolabeled test compound was reported to be 97.9% ascertained by two different thin-layer chromatography systems.

B. Test Animals:

Species: Rat (female)
Strain: Sprague-Dawley CD
Age: Not provided in this report.
Weight (mean): 243.2g ± 2.7 SE (240-265g)
Source: Charles River Breeding Laboratories, Wilmington, MA

III. STUDY DESIGN:

A. Animal Assignment:

Animals were assigned randomly to the following test groups:

Table 1
Animal Assignment in this Study
(Atrazine Distribution Experiment)

Test Group	Daily Oral Dose Given (mg/kg)	Rats (female)	Duration of Exposure (days)
1 Control	0	2	10
2 Low1 (LDT1)	1.0	2	10
3 Mid1 (MDT1)	3.0	2	10
4 Mid2 (MDT2)	7.0	2	10
5 Low3 (MDT3)	10.0	2	10
6 Mid4 (MDT4)	50.0	2	10
7 High (HDT1)	100.0	2	10

[a] After the last oral dose was given, the urinary and fecal levels of radioactivity were measured for 7 days. Animals were individually placed in metabolism cages for the collection of feces and urine. The collection of metabolite was conducted at SRI International. The samples were then shipped to the CIBA-GEIGY laboratory in Greensboro, NC for analysis.

B. Dose Method: The rats were allowed a one-week acclimation period prior to initiation of experimentation. Atrazine was given orally (via a stomach tube) to the rats as an active ingredient or as a radiolabeled active ingredient. The vehicle was 3% corn starch and 0.5% polysorbate 80 (v/v). The rats were allowed free access to animal feed (Purina) and tap water.

C. Statistics:

The following procedures were utilized in analyzing the numerical data:

One- and two-way analysis of variance (ANOVA) was used to assess the statistical significance of results between dose, treatment groups or sex. When appropriate, Dunnetts or Newman-Keuls t-tests were performed to assess differences between group means.

For generating the kinetic models, the excretion data was used. This evaluation was performed by I.W.F. Davidson of Bowman Gray School of Medicine (Wake Forest University). The evaluation was limited because of the low number of rats used in each group. Additional kinetic parameters such as rate constants, half-life values, and alpha and beta distribution values were obtained with the use of the ESTRIP and PCNONLIN computer programs calculated by C.M. Metzler and D.L. Weiner (Statistical Consultants, Edgewood, KY).

D. Quality Assurance:

A signed quality assurance statement was provided by a quality assurance inspector from (1) SRI International, the subcontracting laboratory where the distribution of radiolabeled atrazine was studied and (2) Agrisearch Incorporated, another subcontracting laboratory where the amount of radiolabeled atrazine was measured.

IV. METHODS:

A. Observations: The frequency of clinical observations made on these rats was not provided in this summary report.

Toxicity/mortality (survival) results: There were no treatment-related deaths reported in this study. Rat # 5065 (given 3 mg/kg atrazine for 3 days) favored its right side, and upon examination, the lungs were found to be "present in the lower thoracic area."

B. Experimental Protocol: This experiment was performed to assess in further detail the dose-dependent distribution of atrazine, especially in the red blood cell. As listed in Table 1, in an effort to study in more detail the toxicokinetic disposition of ^{14}C-atrazine as a function of the dose of atrazine and the time of sacrifice, six groups of female Sprague-Dawley rats were treated with ^{14}C-atrazine while another group of female rats served as a control group. The groups of rats were dosed daily for 10 consecutive days at 0 mg/kg (vehicle only), 1 mg/kg, 3 mg/kg, 7 mg/kg, 10 mg/kg, 50 mg/kg, and 100 mg/kg ^{14}C-atrazine. The vehicle was an aqueous solution of corn starch/polysorbate-80.

Urine and feces were collected daily. At 24, 48, 72, 96, 144, 192, 219, 240, 264 and 288 hours, blood samples were obtained via orbital puncture. Five milliliters of blood were collected by aortal puncture at sacrifice. The tissues selected for determining the distribution of ^{14}C-label at each dose are listed in Figure 1. One of the two animals in each group was sacrificed 3 hours after the tenth dose of ^{14}C-atrazine and the other animal in each group was sacrificed 72 hours after the tenth dose of ^{14}C-atrazine. The distribution of ^{14}C-label in the urine, feces, red blood cells, and the following selected tissues was determined for each female rat (Figure 1).

FIGURE 1

	Digestive system		Cardiovascular		Neurological
	Tongue		Aorta*	X	Brain*+@
	Salivary glands*		Heart*@		Peripheral nerve*#
	Esophagus*		Bone marrow*#		Spinal cord (3 levels)*#
	Stomach*		Lymph nodes*	X	Pituitary*
	Duodenum*		Spleen@		Eyes (optic n.)*#
	Jejunum*		Thymus*		
	Ileum*	X	Red blood cell		Glandular
	Cecum*		Urogenital		Adrenal gland*
	Colon*	X	Kidneys*+@		Exorbital lacrimal gland#
	Rectum*		Bladder*	X	Mammary gland*#
X	Liver *+@		Testes*+@		Parathyroids*++
	Gall bladder*#		Epididymides		Thyroids*++
	Pancreas*		Prostate		Other tissues
	Respiratory		Seminal vesicle		Bone (femur)*#
	Trachea*#	X	Ovaries*+@		Muscle*#@
	Lung*@		Uterus*@		Skin*#
	Nose^		Cervix		All gross lesions and masses*
	Pharynx^		Fallopian tubes		Residual Carcass@
	Larynx^				Fat@
					Plasma (blood)@

* Required for subchronic and chronic studies.
^ Required for chronic inhalation.
\# In subchronic studies, examined and preserved only if indicated signs of toxicity or target organ involvement.
\+ Organ weight required in subchronic and chronic studies.
++ Organ weight required for non-rodent studies.
@ Required for determining distribution in metabolism studies.

V. RESULTS:

A. Distribution of radiolabeled atrazine after repeated daily dosing and multiple sampling.

The experiment was conducted with a protocol designed to determine the bodily disposition of ^{14}C-label after exposure for 10 days to a number of doses of ^{14}C-atrazine. The recovery of the total dose averaged 89.2% in rats killed 3 hours after the tenth dose of ^{14}C-atrazine and 94.2% in rats killed 72 hours after the tenth dose of ^{14}C-atrazine averaged. The amount of ^{14}C-label of the total dose excreted in the feces in rats killed at 3 hours was 13.4% and was 14.8% in rats killed at 72 hours independent of the dose. The amount of ^{14}C-label of the total dose excreted in the urine was 69.5% in the rats killed at 3 hours and 76.3% in the rats killed at 72 hours independent of the dose. The total percentage of the initial dose ^{14}C-atrazine excreted in the urine and feces in the rats killed at 3 hours was 82.9% and in the rats killed at 72 hours was 91.1%.

Plasma concentrations of atrazine. In this experiment, plasma concentrations were related linearly to the dose of ^{14}C-atrazine (Table 2). That is, plasma concentrations in rats given 100 mg/kg ^{14}C-atrazine were roughly 100 times that of rats given 1 mg/kg ^{14}C-atrazine. This comparison applies to all of the dosage groups at most time points listed in Table 2. Overall, during daily dosing plasma levels of atrazine or its metabolites generally rose and reached an apparent plateau or steady-state. After daily dosing had stopped the following toxicokinetic values were calculated from the data obtained:

- the whole body half-life, or $t_{1/2}$, of 38.6 hours (1.61 days) for the elimination of atrazine or its metabolites,
- the estimated volume of distribution, or V_d, for the daily dose of 10 mg/kg was 4.15 L/kg, and
- at a dose of 10 mg/kg, the mean plasma concentration of atrazine or its metabolites at steady-state was 5.61 mg-equivalents ^{14}C-label/L of plasma.

For distribution models that follow first-order kinetics such as this model proposed for atrazine, two relationships are reported: (1) $t_{1/2}$ and V_d are independent of the dose and (2) the plasma concentration of ^{14}C-label is directly proportional to the dose of ^{14}C-atrazine.

Table 2
Plasma Levels of ^{14}C-Label (ppm) During the Dosing Period and at Sacrifice
(taken from Table VIII)

Dose	1 mg/kg		3 mg/kg		7 mg/kg		10 mg/kg		50 mg/kg		100 mg/kg	
Rat #:	R5062	R5063	R5064	R5065	R5066	R5067	R5068	R5069	R5070	R5071	R5072	R5073
Hour of Sacrifice:	3	72	72	3	3	72	3	72	3	72	3	72
Time (hrs.):												
24	0.068	0.061	0.063	0.375	0.741	0.562	1.062	1.164	9.291	8.279	27.104	22.298
48	0.186	0.181	0.452	0.615	1.058	1.884	2.009	1.593	7.161	.129?	28.946	23.101
72	0.469	0.405	1.383	1.468	3.267	3.248	4.168	3.957	20.911	17.778	40.320	57.877
96	0.506	0.596	1.808	1.845	3.311	3.747	4.165	4.661	21.249	24.448	52.271	56.580
144	0.582	0.594	2.150	2.608	4.225	3.359	5.069	5.109	25.169	24.604	59.751	69.514
192	0.560	0.658	1.668	1.941	4.066	3.533	5.343	4.725	23.437	27.682	48.671	44.420
219	0.583	--	--	1.406	3.748	--	5.067	--	21.351	--	51.715	--
240	--	0.185	0.703	--	--	1.628	--	3.099	--	26.413	--	29.566
264	--	0.144	0.789	--	--	1.371	--	1.713	--	13.352	--	17.682
288	--	0.117	0.340	--	--	0.868	--	1.600	--	8.302	--	14.775

RBC concentrations of atrazine. The same experimental method used for determining plasma concentrations of atrazine and its metabolites was employed to measure the level of ^{14}C-label in red blood cells (RBCs). The concentration of ^{14}C-label in RBCs rose during repeated daily dosing of ^{14}C-atrazine and did not reach a plateau or steady state (Table 3). RBC concentrations appeared to be proportional (usually superlinear) to the dose of ^{14}C-atrazine. After cessation of daily dosing, the concentration of ^{14}C-label declined for all doses except the highest dose, 100 mg/kg ^{14}C-atrazine.

After daily dosing was stopped, the data was obtained from the level of ^{14}C-label in the urine. The following toxicokinetic values were calculated from those data:

- the mean dosage half-life, or $t_{1/2}$, was 1562.9 hours (8.14 days) for the elimination of atrazine or its metabolites from RBCs,
- the estimated volume of distribution, or V_d, for the daily dose of 10 mg/kg was 0.7 L/kg, and
- at a dose of 10 mg/kg, the mean plasma concentration of atrazine or its metabolites at steady-state was 104.6 mg-equivalents ^{14}C-label/L of cells.

The RBC:plasma concentration ratio was roughly related linearly in all dose levels. The estimated half-life of 8.14 days and the large volume of distribution 104.6 mg-equivalents/L) in RBCs indicate that extensive binding of atrazine and its metabolites in RBCs was occurring. (The life span of a rat RBC is 45-56 days). The author speculates that binding of ^{14}C-label is of a covalent nature.

Table 3
Red Blood Cell Levels of ^{14}C-Label (ppm) During the Dosing Period and at Sacrifice
(taken from Table IX)

Dose	1 mg/kg		3 mg/kg		7 mg/kg		10 mg/kg		50 mg/kg		100 mg/kg	
Rat #:	R5062	R5063	R5064	R5065	R5066	R5067	R5068	R5069	R5070	R5071	R5072	R5073
Hour of Sacrifice:	3	72	72	3	3	72	3	72	3	72	3	72
Time (hrs.):												
24	0.93	0.54	2.57	4.67	5.19	6.39	7.31	7.81	50.87	36.59	109.06	70.38
48	1.48	1.27	4.29	8.48	12.82	14.17	20.72	15.88	124.35	87.00	234.05	190.48
72	2.64	2.77	--	--	19.98	23.08	31.55	26.71	177.56	129.41	292.78	275.59
96	3.51	2.76	21.47	22.01	26.65	30.38	39.07	37.59	225.49	160.46	474.56	305.37
144	5.18	4.24	--	--	37.07	30.78	60.65	53.79	358.75	289.12	881.41	649.45
192	6.61	5.04	23.44	11.63	43.78	50.05	63.84	63.85	415.80	362.30	695.14	529.04
219	10.03	--	--	21.20	54.98	--	83.92	--	307.74	--	517.23	--
240	--	5.98	18.57	--	--	51.45	--	85.63	--	324.18	--	551.40
264	--	5.41	18.11	--	--	46.65	--	67.83	--	318.95	--	605.28
288	--	5.34	18.50	--	--	50.77	--	41.26	--	271.48	--	611.42

Tissue concentrations of atrazine. The tissue concentrations of atrazine and its metabolites were measured in selected tissues from animals killed at 3 and at 72 hours (Table 4). At all doses, tissue levels of ^{14}C-label are consistently lower in all animals killed 72 hours after cessation of ^{14}C-atrazine exposure, a finding that corroborates the observed decline in plasma concentration of ^{14}C-label (Table 2). The liver had the highest tissue concentration of ^{14}C-label, followed by the kidney, pituitary, ovary and brain. The pectoral and inguinal mammary glands had the lowest tissue concentration in this experiment. In respect to making dose comparisons, tissue levels of ^{14}C-label were generally superlinear, i.e., the tissue level in rats given 100 mg/kg ^{14}C-atrazine was generally 200 times higher than that of rats given 1 mg/kg ^{14}C-atrazine. In animals sacrificed at 72 hours, the mammary tissue:plasma concentration ratio at 1 mg/kg was 0.042 and at 100 mg/kg was 0.49; a difference that is roughly proportional to the dose of atrazine.

Table 4
Tissue Levels of ^{14}C-Label (ppm) at Sacrifice
(taken from Table X)

Dose	1 mg/kg		3 mg/kg		7 mg/kg		10 mg/kg		50 mg/kg		100 mg/kg	
Rat #:	R5062	R5063	R5064	R5065	R5066	R5067	R5068	R5069	R5070	R5071	R5072	R5073
Hour of Sacrifice:	3	72	72	3	3	72	3	72	3	72	3	72
Tissue:												
Liver	2.97	2.33	5.40	8.06	16.21	8.05	20.86	12.37	54.87	32.58	102.37	55.88
Pituitary	1.18	0.50	1.67	3.54	7.32	3.24	9.36	4.49	36.91	18.18	71.68	33.90
Ovary	1.14	0.48	1.59	3.62	7.28	2.81	9.08	4.69	36.30	17.42	76.39	33.02
Brain	0.39	0.24	0.90	1.57	3.24	1.55	4.12	2.04	14.59	8.99	30.25	11.84
Kidney	1.36	0.61	1.64	3.54	6.91	3.35	9.88	4.70	29.31	16.73	78.64	26.13
Mammaries:												
Pectoral	0.13	0.05	0.38	0.46	0.52	0.24	1.24	0.75	4.41	3.32	6.30	7.33
Inguinal	0.06	0.05	0.15	0.19	0.79	0.11	0.54	0.65	4.06	2.29	4.77	0.33

V. DISCUSSION

The distribution of atrazine in rats was found to be dose-dependent. Of the tissues studied, the red blood cells store the highest levels of atrazine, apparently through the covalent binding of a metabolite. In rats exposed to a dose of 100 mg/kg atrazine for 10 days, in decreasing order, the levels found in the following tissues were: red blood cell, liver, kidney, ovary, pituitary, brain, pectoral region of the mammaries. Under the exposure regimen used in this study, atrazine does not accumulate in the tissues of the rat, except perhaps for the red blood cell. The pattern of atrazine tissue distribution found in this report was similar that found in male rats exposed to a similar dosage regimen (MRID No. 404313-09, Study No. ABR-85104).

The distribution of atrazine was reported to follow first-order kinetics. Two major relationships are found: (1) the whole body half-life, or $t_{1/2}$, and the volume of distribution, or V_d, are independent of the dose of atrazine and (2) the plasma concentration of atrazine and/or its metabolites are directly proportional to the dose of atrazine. Plasma concentrations of atrazine measured during and after atrazine exposure showed that the whole body half-life ($t_{1/2}$) of atrazine or its metabolites is 38.6 hours (1.61 days) in rats. These reported findings further indicate that atrazine does not accumulate under this exposure regimen in the rat.

Summary. The whole body half-life for atrazine is 1.61 days. The red blood cells store the highest concentration of atrazine in the rat, apparently through the covalent binding of a metabolite. Under the dose regimen employed in this study, atrazine does not accumulate in the rat, except perhaps for the red blood cell.

Classification: Acceptable: This classification is based on the fact that the methodology requirements established in the Pesticide Assessment Guidelines, Subdivision F §85-1 have been satisfied only for reporting the disposition of atrazine in female rats. However, all of the data requirements for metabolism studies set forth in §85-1 have not been reported.

006718

Reviewed by: Sanford W. Bigelow, Ph.D. 5/6/88
Section VI, Toxicology Branch (TS-769C)
Secondary reviewer: Judith W. Hauswirth, Ph.D. Judith W. Hauswirth 5/9/88
Section VI, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

I. SUMMARY:

STUDY TYPE: Metabolism - rat (85-1) CASWELL NO: 63

ACCESSION NUMBER: MRID NO.: 404313-06

TEST MATERIAL: Atrazine

SYNONYMS: 2-Chloro-4-ethylamino-6-isopropylamino-s-triazine

STUDY NUMBER: ABR-87115

SPONSOR: CIBA-GEIGY Corp., Agricultural Division, P.O. Box 18300 Greensboro, NC 27419 Thomas Parshley, Regulatory Specialist (919) 292-7100 X7207

TESTING FACILITY: CIBA-GEIGY Corp., Biochemistry Dept., P.O. Box 18300 Greensboro, NC 27419

TITLE OF REPORT: Characterization and Identification of Atrazine Metabolites From Rat Urine (General Metabolism).

AUTHOR: B.J. Miles

REPORT ISSUED: November 17, 1987

CONCLUSIONS:

The characterization and identification of a number of atrazine metabolites in the female rat was reported in this study. To this end, two experiments were conducted with the use of two groups of rats.

The data reported in this study indicates that dechlorination of the triazine ring and N-dealkylation are the major metabolic pathways for atrazine in rats. Oxidation of the alkyl substituents of atrazine appears to be a minor and secondary metabolic route.

The elimination of atrazine in female rats was also reported in this study. The urinary route accounted for 47.4% of the elimination of atrazine and/or its metabolites whereas 49.3% was eliminated via the fecal route. The tissues contained 5.75% of the atrazine and/or its metabolites while the blood contained the remaining 1.4%. This pattern of excretion differs from male or female rats given repeated oral doses of atrazine, i.e., single oral exposure results in about 50:50 urinary:fecal excretion whereas repeated oral exposure results in about about 75:25 urinary:fecal excretion (see MRID Nos. 404313-05 and 404313-09 for more details). The amount of atrazine and/or its metabolites eliminated via exhalation was not reported. A recovery of 103.78% of the administered radiolabeled atrazine was achieved. The majority of atrazine and/or its metabolites was reported to be excreted via the urine and feces.

Classification: **Acceptable:** This classification is based on the fact that the methodology requirements established in the Pesticide Assessment Guidelines, Subdivision F §85-1 have been satisfied only for reporting the identity of urinary metabolites of atrazine in female rats. However, all of the data requirements for metabolism studies set forth in §85-1 have not been reported, i.e., the urinary and fecal metabolites of atrazine in male rats and the fecal metabolites of atrazine in females must be identified.

II. MATERIALS:

A. Test Compound: Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine)

Description: Not provided in this report.
Batch #: Not provided in this report.
Purity: Not provided in this report for the nonradiolabeled compound.
Radiolabeling procedure:
All carbons in the triazine moiety of atrazine were replaced with carbon-14. The specific activity of the radiolabeled compound was 1.0 microCurie/mg. The purity of the radiolabeled test compound was reported to be ≥ 97%.

B. Test Animals:

Species: Rat (female)
Strain: Sprague-Dawley
Age: Not provided in this report.
Weight (mean): about 0.2 kg
Source: Harlan Sprague-Dawley, Indianapolis, IN

III. STUDY DESIGN:

A. Animal Assignment:

Animals were assigned randomly to the following test groups:

Table 1
Animal Assignment in this Study
(Atrazine Metabolism Experiment)

Test Group	Daily Oral Dose Given (mg/kg)	Rats (female)	Duration of Exposure (day)
1 High	100.0	5	1
2 Mid	16.2 - 19.6	8	1

a After the last oral dose was given, the urinary and fecal levels of radioactivity were measured for 24 hours. Animals were individually placed in metabolism cages for the collection of urine.

B. Dose Method: The rats were allowed a 5-day acclimation period prior to initiation of experimentation. Atrazine was given orally (via a stomach tube) to the rats as an active ingredient or as a radiolabeled active ingredient. The vehicle was 1% methyl carboxymethyl cellulose and Hi-Sil-233 brand of powdered silica used to suspend the atrazine in solution. The rats were allowed free access to animal feed (Purina) and deionized water.

C. Statistics:

No statistical procedures were used in this study.

D. Quality Assurance:

A signed quality assurance statement was provided by a quality assurance inspector from the registrant, the laboratory where the metabolism of radiolabeled atrazine was studied. According to the statement, the Good Laboratory Practice methods were followed in this study. However, this metabolism study was reported not meet the Good Laboratory Practices Requirements of 40 CFR Part 160 because:

(1) "A complete set of biological phase SOPs have not been established.

(2) There was no QA inspection of the study because the QAU was not a fully functional unit at the time the study was conducted.

(3) There was no QA audit of the final report ABR-87115."

IV. METHODS:

A. Observations: The frequency of clinical observations made on these rats was not provided in this summary report.

Toxicity/mortality (survival) results: There were no treatment-related deaths reported in this study.

B. Experimental Protocol: This experiment was conducted to identify the atrazine metabolites in two groups of rats.

As shown in Table 1, one group of female rats was given a single dose of atrazine in an effort to produce sufficient levels of urinary metabolites of atrazine for identification. Five adult female Sprague-Dawley rats (about 0.2 kg) were administered 100 mg/kg ^{14}C-atrazine. Samples of urine and feces were obtained at 24, 48, and 72 hours. After taking samples for 72 hours, the rats were sacrificed and 5 ml of blood and the liver were obtained.

In another group of animals, 8 rats were given a single oral exposure of 16.18 - 19.64 mg/kg ^{14}C-atrazine. Urinary metabolites were collected over a 24-hour period following treatment. The metabolites of atrazine were isolated and identified by the following series of analytical chemistry steps:

(1) charcoal cleanup,

(2) C_{18} Bond-Elut separation,

(3) Aminex A-4 cation exchange column chromatography,

(4) Aminex A-25 anion exchange column chromatography or PRP-1 (reverse-phase) HPLC, and finally

(5) confirmation by comparing to the infrared spectra and mass spectra of authentic synthesized standards.

V. RESULTS:

A. The In Vivo Metabolism of Atrazine.

To examine the metabolism of atrazine in rats, 100 mg/kg of ^{14}C-atrazine was given to rats and the ^{14}C-labeled metabolites were isolated and identified. A recovery of 103.78% of the total radioactivity was achieved. The urinary route accounted for 47.4% of the elimination whereas 49.3% of the ^{14}C-label was eliminated via the fecal route. The tissues contained 5.75% of the ^{14}C-label while the blood contained the remaining 1.4% of the ^{14}C-label. The amount of ^{14}C-label eliminated via exhalation was not reported.

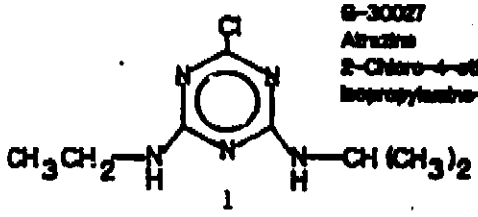
The molecular structures of the urinary metabolites obtained from the first group rats were unattainable, so a second group of 8 rats were given 16.18-19.64 mg/kg ^{14}C-atrazine. The metabolites were collected within the 0 to 24 hour time period after exposure. The urine was freeze dried. Metabolites were then dissolved in a small amount of water that was acidified with HCl to pH 3.0 and separated with an amino acid analyzer (to detect the amino acid residues of glutathione) coupled with a cation exchange column.

A total of 19 radioactive peaks were detected, three of which were identified as metabolites by comparison of the infrared and mass spectra. The identity of two other metabolites was postulated based on additional mass spectral information. The molecular structures of some of the atrazine metabolites are shown in Figure 1 and the numbers in this figure correspond to the metabolites discussed in the text. Only four of these metabolites were identified and were reported, they were:

- 2-hydroxy-atrazine (7),
- 2-hydroxy-4-amino-6-isopropylamino-s-triazine (8),
- 2-hydroxy-4-ethylamino-6-amino-s-triazine (14), and
- 2-hydroxy-4,6-diamino-s-triazine (3).

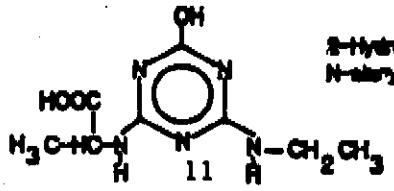
The identification of the four metabolites above indicates that dechlorination of the triazine ring and N-dealkylation are the major metabolic pathways for atrazine in rats. Because several other minor metabolites that possess omega-carboxyl moieties were identified (5, 10, 11, 12),

FIGURE I. CHEMICAL NAMES AND STRUCTURES



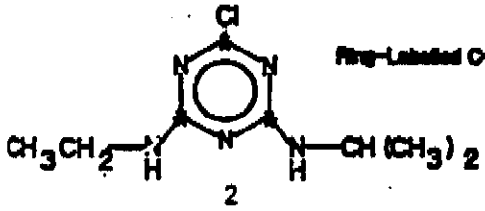
1

G-30027
Atrazine
2-Chloro-4-ethylamino-6-isopropylamino-s-triazine



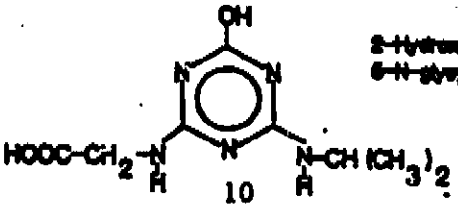
11

2-Hydroxy-4-ethylamino-6-N-alanyl-s-triazine



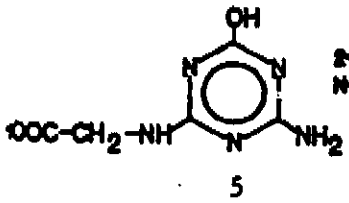
2

Ring-Labeled C-14 Atrazine



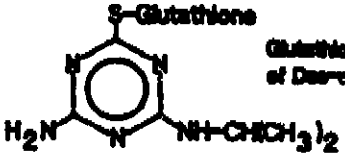
10

2-Hydroxy-4-isopropylamino-6-N-glycyl-s-triazine



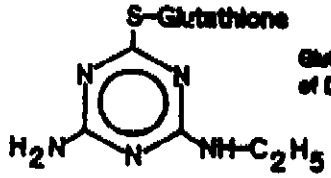
5

2-Hydroxy-4-amino-6-N-glycyl-s-triazine



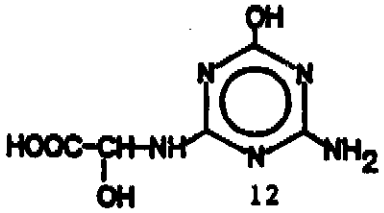
17

S-Glutathione
Glutathione Conjugate of Desethyl atrazine



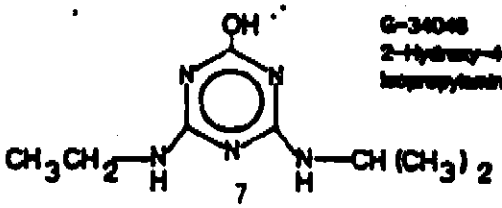
18

S-Glutathione
Glutathione Conjugate of Desisopropyl atrazine



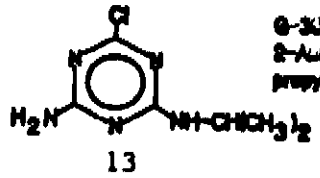
12

2-Hydroxy-4-amino-6-N-(hydroxyglycyl)-s-triazine

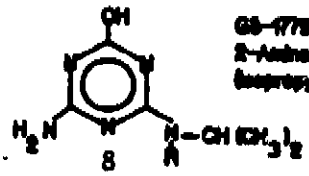


7

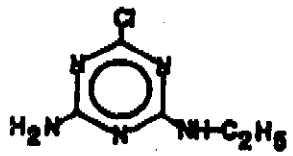
G-34048
2-Hydroxy-4-ethylamino-6-isopropylamino-s-triazine



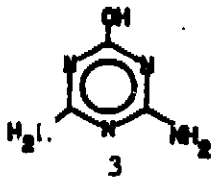
13



8

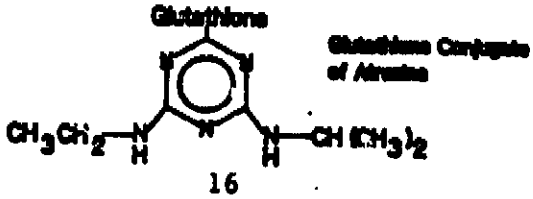


14

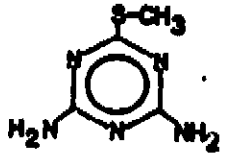


3

15

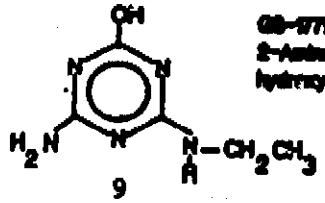


16

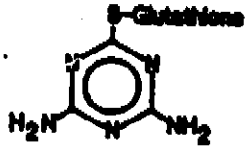


6

4



9



19

FIGURE I. CHEMICAL NAMES AND STRUCTURES (Cont.)

oxidation of the terminal methyl moieties in the alkyl substituents appears to be a minor and secondary metabolic route.

B. The In Vitro Metabolism of Atrazine.

The author of this study offers the results of a published study on atrazine metabolism performed by Dauterman and Muecke (1974. Pesticide Biochemistry and Physiology 4:212-219) in an effort to account for the covalent binding of atrazine in RBCs.

The method published by Dauterman and Muecke is reported as the following steps. Radiolabeled atrazine was incubated with rat liver microsomes with or without the addition of the metabolic cofactors, glutathione and NADPH. Six metabolites were identified by chromatography against synthetic standards. The results corroborate the findings in the in vivo experiment that N-dealkylation is the major metabolic pathway. Also, the isopropyl moiety is hydrolyzed more easily than the ethyl substituent. Conjugation with glutathione was found to occur with most of the atrazine metabolites previously discussed when cytosolic cell fractions were included in the in vitro reactions.

Covalent binding in RBCs. The author argues that the glutathione-containing metabolites of atrazine may be catalyzed by a "carbon-sulfur lyase," an enzyme that cleaves the glutathione residue and leaves a thiol group on the atrazine metabolite. However, the author has not presented evidence whether lyase is present in red blood cells.

V. DISCUSSION:

The characterization and identification of a number of atrazine metabolites in the female rat was reported in this study. To this end, two experiments were conducted with the use of two groups of rats.

The data reported in this study indicates that dechlorination of the triazine ring and N-dealkylation are the major metabolic pathways for atrazine in rats. Oxidation of the alkyl substituents of atrazine appears to be a minor and secondary metabolic route.

The elimination of atrazine in female rats was also reported in this study. The urinary route accounted for 47.4% of the elimination of atrazine and/or its metabolites whereas 49.3% was eliminated via the fecal route. The tissues contained 5.75% of the atrazine and/or its metabolites while the blood contained the remaining 1.4%. This pattern of excretion differs from male or female rats given repeated oral doses of atrazine, i.e., single oral exposure results in about 50:50 urinary:fecal excretion whereas repeated oral exposure results in about about 75:25 urinary:fecal excretion (see MRID Nos. 404313-05 and 404313-09 for more details). The amount of atrazine and/or its metabolites eliminated via exhalation was not reported. A recovery of 103.78% of the administered radiolabeled atrazine was achieved. The majority of atrazine and/or its metabolites was reported to be excreted via the urine and feces.

Classification: Acceptable: This classification is based on the fact that the methodology requirements established in the Pesticide Assessment Guidelines, Subdivision F §85-1 have been satisfied only for reporting the identity of urinary metabolites of atrazine in female rats. However, all of the data requirements for metabolism studies set forth in §85-1 have not been reported, i.e., the urinary and fecal metabolites of atrazine in male rats and the fecal metabolites of atrazine in females must be identified.

006718

Reviewed by: Sanford W. Bigelow, Ph.D. 5/6/88
Section VI, Toxicology Branch (TS-769C)
Secondary reviewer: Judith W. Hauswirth, Ph.D. Judith W. Hauswirth 5/9/88
Section VI, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

I. SUMMARY:

STUDY TYPE: Metabolism - rat (85-1) CASWELL NO: 63

ACCESSION NUMBER: MRID NO.: 404313-09

TEST MATERIAL: Atrazine

SYNONYMS: 2-Chloro-4-ethylamino-6-isopropylamino-s-triazine

STUDY NUMBER: ABR-85104

SPONSOR: CIBA-GEIGY Corp., Agricultural Division, P.O. Box 18300 Greensboro, NC 27419 Thomas Parshley, Regulatory Specialist (919) 292-7100 X7207

TESTING FACILITY: CIBA-GEIGY Corp., Biochemistry Dept., P.O. Box 18300 Greensboro, NC 27419

TITLE OF REPORT: Metabolism of ^{14}C-Atrazine in Orally Dosed Rats (General Metabolism).

AUTHOR: B.J. Simoneaux

REPORT ISSUED: December 6, 1985

CONCLUSIONS:

This report is a balance study of the disposition of atrazine in male rats repeatedly orally exposed to this agent. Atrazine appears to be rapidly excreted in the rat under these exposure conditions. About 95% of the administered dose is eliminated within 18 days after the last atrazine exposure. The urinary route accounted for about 70% of the elimination whereas about 25% was eliminated via the fecal route. The RBCs store the highest levels followed by the liver, kidney and brain. Under these exposure conditions, atrazine does not accumulate in the rat. The total recovery of administered radiolabeled atrazine for the high and low dose groups was 93.4% and 103.9%, respectively.

Atrazine appears to be rapidly excreted in the rat under these exposure conditions. About 95% of the administered dose is eliminated within 18 days after the last atrazine exposure. For the low and high dose groups of rats, respectively, the urinary route accounted for 72.7% and 67.2% of the elimination while 27.8% and 23.9% of the atrazine and/or its metabolites were eliminated via the fecal route. Elimination of atrazine and/or its metabolites by way of exhalation was not monitored or reported.

The tissues contained the remaining amount of the atrazine and/or its metabolites. The peak tissue levels in the low dose group occurred at 10 days whereas the peak levels in the high dose group was reported at 8 days. The highest tissue levels in the low dose group (0.1 mg/rat) were found at 10 days in the RBC followed by liver, kidney and brain. In decreasing order, the highest tissue levels of atrazine in the high dose group of rats (1.0 mg/rat) at 8 days were: RBC, liver, kidney and brain. In general, 10 days after the last dose of atrazine (at the 18-day sacrifice), the RBCs, liver, kidney and brain had minimal levels (about 1%) of atrazine and/or its metabolites remaining. Under these exposure conditions, atrazine does not accumulate in these tissues in rats repeatedly exposed to atrazine. The pattern of atrazine tissue distribution found in this report was similar that found in female rats exposed to a similar dosage regimen (MRID No. 404313-05, Study No. ABR-87087).

Classification: Acceptable: This classification is based on the fact that the methodology requirements established in the Pesticide Assessment Guidelines, Subdivision F §85-1 have been satisfied only for reporting the distribution and excretion of atrazine in male rats. However, all of the data requirements for metabolism studies set forth in §85-1 have not been reported.

II. MATERIALS:

A. Test Compound:

Description: Atrazine
Batch #: Not reported in this study
Purity: Not provided in this summary report for the nonradiolabeled compound.
Radiolabeling procedure:
All carbons in the triazine moiety of atrazine were replaced with carbon-14. The specific activity of the radiolabeled compound were 13.5 microCuries/mg and 12.9 microCuries/mg for the low and high dose groups, respectively. The purity of the radiolabeled test compound was reported to be $\geq$ 97.5% ascertained by a thin-layer chromatography system.

B. Test Animals:

Species: Rat (male)
Strain: Harlan Sprague-Dawley
Age: Not provided in this report.
Weight (mean): 250g
Source: Harlan Madison, WI

III. STUDY DESIGN:

A. Animal Assignment:

Animals were assigned randomly to the following test groups:

Table 1
Animal Assignment in this Study
(Atrazine Metabolism Experiment)

Test Group	Daily Oral Dose Given[a] (mg/kg)	Rats (male)	Day of Sacrifice	Duration of Exposure (days)
2 Low	0.4	3	5	4
Low	0.4	3	7	7
Low	0.4	3	9	7
Low	0.4	3	10	7
Low	0.4	3	14	7
Low	0.4	3	18	7
3 High	4.0	3	5	4
High	4.0	3	7	7
High	4.0	3	9	7
High	4.0	3	10	7
High	4.0	3	14	7
High	4.0	3	18	7

[a] After the last oral dose was given, the urinary and fecal levels of radioactivity were measured at 24-hour intervals in the group of rats exposed for 18 days. Animals were individually placed in metabolism cages for the collection of feces and urine. There was no control group.

B. Dose Method: Atrazine was given orally (via a stomach tube) to the rats as a radiolabeled active ingredient. The 250 g rats were given 0.1 mg/rat (low dose) or 1.0 mg/rat (high dose). The vehicle was in the aqueous Carbowax-200 (PEG 200) formulation (0.3 ml ethanol:0.2 ml water:0.5 ml PEG 200). The rats were allowed free access to animal feed and tap water.

C. Statistics:

The following procedure was utilized in analyzing the numerical data:

The SOP method of Wolf and Sumner, AG-276, "Statistical methods in the measurement of radioactivity" were used to calculate ppm-equivalents of the ^{14}C-label obtained from the rats.

D. Quality Assurance:

A signed quality assurance statement was provided by a quality assurance inspector from the registrant, the laboratory where the metabolism of radiolabeled atrazine was studied. According to the statement, the Good Laboratory Practice methods were followed in this study.

IV. METHODS:

A. Observations: The frequency of clinical observations made on these rats was not provided in this study.

Toxicity/mortality (survival) results: There were no treatment-related deaths reported in this study.

B. Experimental Protocol: The procedure was conducted to assess the metabolism of atrazine.

Three male rats in each group were repeatedly dosed and then sacrificed 5, 7, 9, 10, 14, and 18 days after dosing was initiated (for details, see Table 1). Urine and feces were collected for analysis from the rats exposed for the 18-day period. The rats were sacrificed and the following selected tissues were analyzed for ^{14}C content (Figure 1).

FIGURE 1

	Digestive system
	Tongue
	Salivary glands*
	Esophagus*
X	Stomach*
X	Duodenum*
X	Jejunum*
X	Ileum*
	Cecum*
X	Colon*
	Rectum*
X	Liver *+@
	Gall bladder*#
	Pancreas*
	Respiratory
	Trachea*#
	Lung*@
	Nose^
	Pharynx^
	Larynx^

	Cardiovascular
	Aorta*
	Heart*@
	Bone marrow*#
	Lymph nodes*
	Spleen@
	Thymus*
X	Red blood cell
	Urogenital
X	Kidneys*+@
	Bladder*
	Testes*+@
	Epididymides
	Prostate
	Seminal vesicle
	Ovaries*+@
	Uterus*@
	Cervix
	Fallopian tubes

	Neurological
X	Brain*+@
	Peripheral nerve*#
	Spinal cord (3 levels)*#
	Pituitary*
	Eyes (optic n.)*#
	Glandular
	Adrenal gland*
	Exorbital lacrimal gland#
	Mammary gland*#
	Parathyroids*++
	Thyroids*++
	Other tissues
	Bone (femur)*#
X	Muscle*#@
	Skin*#
	All gross lesions and masses*
X	Residual Carcass@
X	Fat@
X	Plasma (blood)@

* Required for subchronic and chronic studies.
^ Required for chronic inhalation.
\# In subchronic studies, examined and preserved only if indicated signs of toxicity or target organ involvement.
\+ Organ weight required in subchronic and chronic studies.
++ Organ weight required for non-rodent studies.
@ Required for determining distribution in metabolism studies.

[FIFRA Subdivision F test guidelines §85-1 (e)(3)(i) require that, in addition to the tissues listed in Figure 1 above, the levels of atrazine or its metabolites shall be measured in the testes, heart, lung, spleen and uterus.]

V. RESULTS:

B. The Metabolism of Atrazine

To examine the metabolism of atrazine in rats, two doses were employed, 0.4 and 4.0 mg/kg of ^{14}C-atrazine was given to rats and the ^{14}C-label was measured in selected tissues and in the rats exposed for 18-days, urinary and fecal levels of ^{14}C-label were monitored. A recovery of 103.9% and 93.4% was found for the low and high dose groups, respectively. For the low and high dose groups, respectively, the urinary route accounted for 72.74% and 67.2% of the elimination whereas 27.79% and 23.92% of the ^{14}C-label was eliminated via the fecal route. The author reports that about 95% of the administered dose is eliminated within 48 hours after the last exposure.

The tissues contained the remainder of the ^{14}C-label (Tables 2 and 3). The highest tissue levels in the low dose group (0.1 mg/rat) were found at 10 days in the RBC (1.95 ppm) followed by liver (1.10 ppm), kidney (0.74 ppm) and brain (0.38 ppm) and are listed in Table 2. The highest tissue levels of ^{14}C-label, in decreasing order, in the high dose group of rats at 8 days were found as such: RBC (21.66 ppm), liver (6.40 ppm), kidney (5.28 ppm) and brain (2.48 ppm). In general, 10 days after the last dose of ^{14}C-atrazine (at the 18-day sacrifice), the RBCs, liver, kidney and brain had minimal levels (about 1%) of ^{14}C-label remaining. The remaining tissues had lower levels of ^{14}C-label at 8 or 10 days and lower levels remaining at 18 days. The peak tissue levels in the low dose group occurred at 10 day whereas the peak levels in the high dose group was reported at 8 days.

As percentage of administered dose (Table 3), the muscle had the highest levels followed by the liver and RBC. Percentage of tissue levels were highest in those rats sacrificed 4 days after initial atrazine exposure (Table 3).

Table 2
Tissue Levels of ^{14}C-Label (ppm) Remaining After Sacrifice
(taken from Table IV)

0.1 mg/rat	Time of Sacrifice (Days)					
	4	6	8	10	14	18
Plasma	0.06	0.05	0.06	0.04	0.01	0.01
RBC	1.11	1.18	1.63	1.95	1.31	1.53
Fat	0.04	0.04	0.04	0.05	0.05	0.03
Brain	0.29	0.30	0.39	0.38	0.27	0.24
Muscle	0.13	0.13	0.15	0.15	0.12	0.11
Kidney	0.67	0.63	0.74	0.71	0.32	0.23
Liver	0.88	0.91	1.06	1.10	0.56	0.40
Stomach	0.20	0.66	0.21	0.18	0.10	0.10
Small Intestine	0.21	0.24	0.26	0.15	0.06	0.09
Large Intestine	6.17	0.25	0.20	0.16	0.07	0.09
Large Intestinal Content	0.80	0.87	0.64	0.30	0.11	0.12
4.0 mg/rat						
Plasma	0.55	0.82	1.02	0.37	0.14	0.07
RBC	9.30	16.34	21.66	17.17	15.50	13.78
Fat	0.21	0.28	0.43	0.23	0.15	0.19
Brain	1.23	2.10	2.48	1.76	1.39	1.14
Muscle	0.77	1.27	1.56	0.91	0.83	0.75
Kidney	3.09	4.49	5.28	3.41	1.92	1.26
Liver	4.26	5.48	6.40	4.48	2.87	1.80
Stomach	1.46	2.01	1.96	0.52	0.32	0.15
Small Intestine	1.44	1.87	2.22	0.75	0.32	0.22
Large Intestine	1.28	1.56	1.86	0.97	0.60	0.56
Large Intestinal Content	9.15	12.27	11.94	1.28	7.97	0.22

Table 3
Percent of Dose of ^{14}C-Label Remaining After Sacrifice
(taken from Table III)

0.1 mg/rat	Time of Sacrifice (Days)					
	4	6	8	10	14	18
Plasma	0.15	0.08	0.10	0.05	0.02	0.01
RBC	1.60	1.28	1.39	1.38	1.08	1.11
Fat	0.26	0.20	0.09	0.37	0.16	0.09
Brain	0.12	0.08	0.09	0.08	0.06	0.05
Muscle	3.66	2.65	2.51	2.05	1.81	1.55
Kidney	0.34	0.26	0.23	0.17	0.10	0.06
Liver	2.55	1.94	1.70	1.28	0.85	0.43
Stomach	0.15	0.35	0.10	0.05	0.03	0.02
Small Intestine	0.15	0.18	0.22	0.07	0.03	0.02
Large Intestine	0.04	0.06	0.03	0.02	0.01	0.01
Large Intestinal Content	0.25	0.29	0.13	0.07	0.02	0.02
4.0 mg/rat						
Plasma	0.15	0.13	0.12	0.05	0.02	0.01
RBC	1.48	1.51	1.46	1.38	1.08	0.94
Fat	0.16	0.12	0.13	0.08	0.04	0.06
Brain	0.05	0.06	0.04	0.04	0.03	0.02
Muscle	2.42	2.23	2.04	1.41	1.12	1.00
Kidney	0.18	0.13	0.11	0.09	0.05	0.03
Liver	1.27	0.87	0.72	0.72	0.42	0.25
Stomach	0.08	0.06	0.10	0.03	0.02	0.01
Small Intestine	0.19	0.10	0.09	0.05	0.02	0.01
Large Intestine	0.04	0.02	0.02	0.02	0.01	0.01
Large Intestinal Content	0.38	0.36	0.13	0.04	0.18	0.01

VI. DISCUSSION

This report is a balance study of the disposition of atrazine in male rats repeatedly orally exposed to this agent. Atrazine appears to be rapidly excreted in the rat under these exposure conditions. About 95% of the administered dose is eliminated within 18 days after the last atrazine exposure. The urinary route accounted for about 70% of the elimination whereas about 25% was eliminated via the fecal route. The RBCs store the highest levels followed by the liver, kidney and brain. Under these exposure conditions, atrazine does not accumulate in the rat. The total recovery of administered radiolabeled atrazine for the high and low dose groups was 93.4% and 103.9%, respectively.

Atrazine appears to be rapidly excreted in the rat under these exposure conditions. About 95% of the administered dose is eliminated within 18 days after the last atrazine exposure. For the low and high dose groups of rats, respectively, the urinary route accounted for 72.7% and 67.2% of the elimination while 27.8% and 23.9% of the atrazine and/or its metabolites were eliminated via the fecal route. Elimination of atrazine and/or its metabolites by way of exhalation was not monitored or reported.

The tissues contained the remaining amount of the atrazine and/or its metabolites. The peak tissue levels in the low dose group occurred at 10 days whereas the peak levels in the high dose group was reported at 8 days. The highest tissue levels in the low dose group (0.1 mg/rat) were found at 10 days in the RBC followed by liver, kidney and brain. In decreasing order, the highest tissue levels of atrazine in the high dose group of rats (1.0 mg/rat) at 8 days were: RBC, liver, kidney and brain. In general, 10 days after the last dose of atrazine (at the 18-day sacrifice), the RBCs, liver, kidney and brain had minimal levels (about 1%) of atrazine and/or its metabolites remaining. Under these exposure conditions, atrazine does not accumulate in these tissues in rats repeatedly exposed to atrazine. The pattern of atrazine tissue distribution found in this report was similar that found in female rats exposed to a similar dosage regimen (MRID No. 404313-05, Study No. ABR-87087).

Classification: Acceptable: This classification is based on the fact that the methodology requirements established in the Pesticide Assessment Guidelines, Subdivision F §85-1 have been satisfied only for reporting the distribution and excretion of atrazine in male rats. However, all of the data requirements for metabolism studies set forth in §85-1 have not been reported.

006718

Reviewed by: Sanford W. Bigelow, Ph.D. 5/10/88
Section VI, Toxicology Branch (TS-769C)
Secondary reviewer: Robert P. Zendzian, Ph.D. 5/11/88
Tertiary Reviewer: Judith W. Hauswirth, Ph.D. Judith W. Hauswirth 5/11/88
Section VI, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

I. **SUMMARY:**

STUDY TYPE: Dermal Absorption - rat (85-3) CASWELL NO: 63

ACCESSION NUMBER: MRID NO.: 404313-10

TEST MATERIAL: Atrazine

SYNONYMS: 2-Chloro-4-ethylamino-6-isopropylamino-s-triazine

STUDY NUMBER: ABR-83081

SPONSOR: CIBA-GEIGY Corp., Agricultural Division, P.O. Box 18300 Greensboro, NC 27419 Thomas Parshley, Regulatory Specialist (919) 292-7100 X7207

TESTING FACILITY: CIBA-GEIGY Corp., Biochemistry Dept., P.O. Box 18300 Greensboro, NC 27419

TITLE OF REPORT: Excretion Rate of ^{14}C-Atrazine From Dermally Dosed Rats (General Metabolism).

AUTHOR: G.J. Marco

REPORT ISSUED: October 20, 1983

CONCLUSIONS:

This report describes a dermal penetration study of atrazine in rats. The percentage of dermally-applied atrazine that was excreted in the urine or feces was reported to be dependent on the atrazine dose (0.025, 0.25, 2.5 or 5.0 mg/kg). Urinary excretion was the major route of elimination that ranged from 36.9% in rats dermally-exposed to 0.25 mg/kg to 58.8% in rats exposed to 5.0 mg/kg atrazine. Of the atrazine that was absorbed through the skin, about 75% of the atrazine and/or its metabolites were eliminated in the urine or feces within 72 hours after dermal exposure.

The pattern of urinary and fecal excretion was similar for the 4 dosages: cumulative urinary and fecal levels of atrazine or its metabolites were found to plateau at about 120 hours after dermal exposure. Surprisingly, the percent of dose that was excreted in the urine and feces increased with an increase in

dosage. A small percentage of the dermally-applied atrazine remained on the skin and was present in skin washes. Lower percentages of the dermally-applied dose were found retained in the skin and in skin washes in those rats given higher doses of atrazine.

Assuming that (1) no atrazine was lost from the dermal application procedure and that (2) the percentage of atrazine unrecovered after 144 hours of measurement was distributed in the rat, 60% to 80% of the dermally-applied dose of atrazine was absorbed through the skin of the rat within 144 hours of exposure.

Classification: Unacceptable: This classification is based on the following reasons:

1) The solvent tetrahydrofuran was used to dissolve atrazine for dermal application. It is not stipulated by the registrant whether tetrahydrofuran is the solvent used for field application. Since the dermal absorption of a compound is dependent on the solvent, the use of an inappropriate solvent will yield irrelevant data regarding dermal absorption.

2) The dermal site of application was not covered by a material that prevents the test substance from exfoliating, or flaking off the skin (a canopy that does not come in contact with skin). Exfoliation of the test compound would both decrease the amount available for absorption and contaminate the urine and feces.

3) Only a few tissues, not the complete set of tissues, were excised and saved for future measurement of the levels of atrazine and its metabolites as required by the recommended testing procedure. Furthermore, the distribution of dermally-applied atrazine in these selected tissues and residual carcass were not investigated in this study.

4) Individual animal data were not reported.

5) In general, for most compounds, the quantity of test substance absorbed will increase with increasing dose, however, the percent of dose absorbed should decrease with increasing dose. This relationship was not found in this study. This anomaly may be the result of using tetrahydrofuran as the vehicle.

II. MATERIALS:

A. Test Compound: Atrazine

Description: Not provided in this study.
Batch #: Not provided in this study.
Purity: Not provided in this study for the nonradiolabeled compound.
Radiolabeling procedure:
All carbons in the s-triazine moiety of atrazine were replaced with carbon-14. The specific activity of the radiolabeled compound was 17.2 microCuries/mg. The purity of the radiolabeled test compound was not reported. The radiolabeled compound was dissolved in tetrahydrofuran.

B. Test Animals:

Species: Rat (female)
Strain: Harlan Sprague-Dawley
Age: Not reported in this study.
Weight: About 200g
Source: Harlan Sprague-Dawley Madison, WI.

III. STUDY DESIGN:

A. Animal Assignment:

Animals were assigned randomly to the following test groups:

Table 1
Animal Assignment in this Study
(Atrazine Metabolism Experiment)

Test Group	Single Dose Given[a] (mg/kg)	Rats (female)	Day of Sacrifice
1 Low	0.025	2	6
2 Mid	0.25	2	6
3 Mid	2.5	2	6
4 High	5.0	2	6

[a] After the last dose was given, the urinary and fecal levels of radioactivity were measured at 24-hour intervals for 6 days. Animals were individually placed in metabolism cages for the collection of feces and urine.

006718

B. Dose Method: Atrazine was given dermally to the rats as a radiolabeled active ingredient dissolved in tetrahydrofuran. The concentration of ^{14}C-atrazine in the tetrahydrofuran was not reported. The rats were allowed free access to animal feed and tap water.

C. Statistics:

No statistical procedures were utilized as reported in this study.

D. Quality Assurance:

A signed quality assurance statement was provided by a quality assurance inspector from the registrant, the laboratory where the metabolism of radiolabeled atrazine was studied. According to the statement, the Good Laboratory Practice methods were not applicable to this study.

IV. **METHODS:**

A. Observations: The frequency of clinical observations made on these rats was not provided in this study.

Toxicity/mortality (survival) results: There were no treatment-related deaths reported in this study.

B. Experimental Protocol: The procedure was conducted to assess the metabolism of atrazine. The 200 g rats were given 0.025, 0.25, 2.5 or 5.0 mg/kg ^{14}C-atrazine.

> "The dorsal hair of [the] rats was shaved with an electric clipper using a surgical shaving head. Only the rats with their stratum corneum intact were selected for treatment. A 1.5 square cm. area was marked on the shaved area and between 15 and 20 ul [(microliter)] of the apropriate [sic] solution containing the ^{14}C-atrazine was applied. The treated area on the rat's back was selected to minimize possible oral ingestion of the compound and the rear of the rats were shackled with short length jewelry chain to prevent scratching of the treated area."

Urine and feces were collected for analysis from the rats exposed for the 18-day period.

The rats were sacrificed and the following selected tissues were collected "and stored for future use if needed" (Figure 1). The treated area of skin was washed with 100 ml of tetrahydrofuran for 24 hours to determine the unabsorbed percentage of the ^{14}C-atrazine. The washed skin was then placed in tissue solubilizer to determine levels of ^{14}C-atrazine absorbed into the skin.

FIGURE 1

TISSUES EXAMINED IN THIS STUDY

X	Heart	X	Spleen	X	Kidneys
X	Liver	X	Lung	X	Residual Carcass

V. **RESULTS:**

The percentage of dermally-applied ^{14}C-atrazine that was excreted in the urine or feces was reported to be dependent on the ^{14}C-atrazine dose. Urinary excretion was the major route of elimination and ranged from 36.9% in rats dermally-exposed to 0.25 mg/kg ^{14}C-atrazine to 58.8% in rats exposed to 5.0 mg/kg ^{14}C-atrazine (Table 1). Most of the ^{14}C-label was eliminated in the urine or feces within 48 hours after dermal exposure (Table 2). The pattern of urinary and fecal excretion, (i.e., comparing the micrograms per square centimeter of dermally-applied ^{14}C-atrazine to the micrograms of excreted ^{14}C-label) was similar for the 4 dosages: urinary and fecal levels of ^{14}C-label were found to plateau at about 120 hours.

A small percentage of the dermally-applied ^{14}C-atrazine remained on the skin and was present in skin washes. About 10% of the ^{14}C-label was recovered in the skin wash from the rats treated with 0.025 mg/kg ^{14}C-atrazine, about 5% was recovered in the 0.25 mg/kg group, almost 4% in the 2.5 mg/kg group, and less than 1% was found from rats treated with 5.0 mg/kg ^{14}C-atrazine. The percentage of the dermally-applied dose retained in the skin was (at mg/kg dose): 0.72% (0.25), 0.56% (0.25), 0.16% (2.5), and 0.11% (5.0). Lower percentages of the dermally-applied dose were found retained in the skin and in skin washes in those rats given higher doses of ^{14}C-atrazine.

Assuming that (1) no ^{14}C-atrazine was lost from the dermal application procedure through exfoliation of the skin and that (2) the percentage of ^{14}C-atrazine unrecovered after 144 hours of measurement was still distributed in the rat:

- in the rats treated with 0.025 mg/kg ^{14}C-atrazine had a total of about 60% of the ^{14}C-label absorbed through the skin,
- rats given 0.25 mg/kg ^{14}C-atrazine absorbed about 76% of the dermally applied dose,
- the group that received 2.5 mg/kg ^{14}C-atrazine absorbed around 76% of the dose, and
- the group of rats administered 5.0 mg/kg ^{14}C-atrazine absorbed about 80% of the dermally-applied dose

as measured at 144 hours after exposure.

Table 2
Percent of Dose of ^{14}C-Label After Dermal Exposure
(taken from Table I)

	Dosage Level (mg/kg)	Percent of Dose After Treatment (hours)						
		24	48	72	96	120	144	Total
Urine	0.025	16.5	10.7	4.1	2.5	1.4	1.6	36.9
	0.25	12.8	10.3	9.9	8.2	4.9	4.2	50.3
	2.5	21.6	15.9	6.8	5.1	3.0	3.2	55.7
	5.0	31.5	13.4	6.5	3.7	2.1	1.8	58.8
Feces	0.025	4.3	5.4	2.4	1.2	0.7	0.9	14.9
	0.25	2.1	5.7	4.0	3.6	2.3	1.9	19.6
	2.5	2.5	9.7	3.3	2.4	1.4	1.2	20.5
	5.0	8.1	5.9	1.8	2.8	1.2	0.75	20.6
Total Excreta	0.025	20.7	16.1	6.6	3.7	2.1	2.5	51.8
	0.25	14.8	16.1	13.9	11.8	7.2	6.0	69.9
	2.5	24.1	25.6	10.1	7.5	4.4	4.4	71.8
	5.0	39.6	19.2	8.3	6.5	3.3	2.6	79.5

Table 3
Levels of ^{14}C-Label (micrograms) After Dermal Exposure
(taken from Table II)

	Dosage Level (mg/kg)	mg	Micrograms of Radiolabel After Treatment (hours)							
			mg/cm^2	24	48	72	96	120	144	Total
Urine	0.038	0.007	0.004	1.2	0.8	0.3	0.2	0.1	0.1	2.8
	0.25	0.05	0.033	6.4	5.1	5.1	4.1	2.4	2.1	25.1
	2.43	0.48	0.32	105.6	77.6	66.1	25.1	29.5	15.7	319.7
	4.9	0.98	0.65	309.5	131.5	64.4	36.5	20.5	18.1	580.6
Feces	0.038	0.007	0.004	0.3	0.4	0.2	0.1	0.1	0.1	1.2
	0.24	0.05	0.033	1.0	2.8	2.0	1.8	1.1	0.9	9.7
	2.43	0.48	0.32	12.0	47.3	16.4	11.7	6.9	11.9	106.3
	4.9	0.98	0.65	79.5	57.7	17.8	27.9	12.1	7.3	202.3
Total Excreta	0.038	0.007	0.004	1.6	1.2	0.6	0.3	0.2	0.2	4.0
	0.24	0.05	0.033	7.4	8.0	7.1	5.9	3.6	3.0	34.9
	2.43	0.48	0.32	117.6	124.9	82.5	36.8	36.4	27.6	426.0
	4.9	0.98	0.65	389.0	189.1	82.2	64.4	32.7	25.5	783.0

VI. DISCUSSION:

This report describes a dermal penetration study of atrazine in rats. The percentage of dermally-applied atrazine that was excreted in the urine or feces was reported to be dependent on the atrazine dose (0.025, 0.25, 2.5 or 5.0 mg/kg). Urinary excretion was the major route of elimination that ranged from 36.9% in rats dermally-exposed to 0.25 mg/kg to 58.8% in rats exposed to 5.0 mg/kg atrazine. Of the atrazine that was absorbed through the skin, about 75% of the atrazine and/or its metabolites were eliminated in the urine or feces within 72 hours after dermal exposure.

The pattern of urinary and fecal excretion was similar for the 4 dosages: cumulative urinary and fecal levels of atrazine or its metabolites were found to plateau at about 120 hours after dermal exposure. Surprisingly, the percent of dose that was excreted in the urine and feces increased with an increase in dosage. A small percentage of the dermally-applied atrazine remained on the skin and was present in skin washes. Lower percentages of the dermally-applied dose were found retained in the skin and in skin washes in those rats given higher doses of atrazine.

Assuming that (1) no atrazine was lost from the dermal application procedure and that (2) the percentage of atrazine unrecovered after 144 hours of measurement was distributed in the rat, 60% to 80% of the dermally-applied dose of atrazine was absorbed through the skin of the rat within 144 hours of exposure.

Classification: Unacceptable: This classification is based on the following reasons:

1) The solvent tetrahydrofuran was used to dissolve atrazine for dermal application. It is not stipulated by the registrant whether tetrahydrofuran is the solvent used for field application. Since the dermal absorption of a compound is dependent on the solvent, the use of an inappropriate solvent will yield irrelevant data regarding dermal absorption.

2) The dermal site of application was not covered by a material that prevents the test substance from exfoliating, or flaking off the skin (a canopy that does not come in contact with skin). Exfoliation of the test compound would both decrease the amount available for absorption and contaminate the urine and feces.

3) Only a few tissues, not the complete set of tissues, were excised and saved for future measurement of the levels of atrazine and its metabolites as required by the recommended testing procedure. Furthermore, the distribution of dermally-applied atrazine in these selected tissues and residual carcass were not investigated in this study.

4) Individual animal data were not reported.

5) In general, for most compounds, the quantity of test substance absorbed will increase with increasing dose, however, the <u>percent</u> of dose absorbed should decrease with increasing dose. This relationship was not found in this study. This anomaly may be the result of using tetrahydrofuran as the vehicle.

006718

Reviewed by: Sanford W. Bigelow, Ph.D. 5/6/88
Section VI, Toxicology Branch (TS-769C)
Secondary reviewer: Judith W. Hauswirth, Ph.D. Judith W Hauswirth 5/9/88
Section VI, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

I. SUMMARY:

STUDY TYPE: Metabolism - rat (85-1) CASWELL NO: 63

ACCESSION NUMBER: MRID NO.: 404313-04

TEST MATERIAL: Atrazine

SYNONYMS: 2-Chloro-4-ethylamino-6-isopropylamino-s-triazine

STUDY NUMBER: ABR-87048

SPONSOR: CIBA-GEIGY Corp., Agricultural Division, P.O. Box 18300 Greensboro, NC 27419 Thomas Parshley, Regulatory Specialist (919) 292-7100 X7207

TESTING FACILITY: CIBA-GEIGY Corp., Biochemistry Dept., P.O. Box 18300 Greensboro, NC 27419
-and-
SRI International, 333 Ravenswood Ave., Mnelo Park, CA 94025 (Study No. LSC-1469)

TITLE OF REPORT: Disposition of Atrazine in the Rat (General Metabolism).

AUTHOR: G.R. Orr

REPORT ISSUED: October 23, 1987

CONCLUSIONS:

The distribution of atrazine in rats was found to be dose-dependent and independent of sex. Of the tissues studied, the red cells store the highest levels of atrazine, apparently through the covalent binding of a metabolite. In rats given a single oral dose of 100 mg/kg atrazine, in decreasing order, the levels found in the following tissues were: red blood cells, heart, spleen, lung, liver, kidney, brain, gonads, pituitary, muscle, bone, fat, and plasma. In rats given repeated daily oral doses of 1 mg/kg atrazine, in decreasing order, the levels found in the following tissues were: red blood cells, liver, spleen, kidney, lung, heart, pituitary, brain, gonads, muscle, bone, fat, and plasma. Under the exposure regimen used in this study, atrazine does not accumulate in the tissues of the rat, except perhaps for the red blood cell. The pattern of atrazine

tissue distribution for atrazine in this study is similar to that found in rats repeated exposed to atrazine (MRID Nos. 404313-05 and 404313-09).

The distribution of atrazine was reported to follow first-order kinetics. Two major relationships are found: (1) the whole body half-life, or $t_{1/2}$, and the volume of distribution, or V_d, are independent of the dose of atrazine and (2) the plasma concentration of atrazine or its metabolites are directly proportional to the dose of atrazine. Plasma concentrations of atrazine measured during and after atrazine exposure showed that the whole body half-life ($t_{1/2}$) of atrazine or its metabolites is 31.3 hours (1.3 days) in rats. These reported findings further indicate that atrazine does not accumulate under this exposure regimen in the rat.

Excretion of atrazine in rats. About 95% of the atrazine administered orally is excreted within 7 days after cessation of exposure. The route of atrazine excretion was reported to be independent of the dose and sex of rat. About 75% of the atrazine is excreted through the urinary route whereas about 20% of the atrazine is eliminated in the feces. The elimination route for the remaining 5% was not reported. Also, the level of atrazine elimination by exhalation or through the skin (sweating) was not reported.

Summary. The whole body half-life of 1.30 days for atrazine is consistent with the observation that about 95% of the administered dose is eliminated within 7 days after exposure. The red cells store the highest concentration of atrazine in the rat, apparently through the covalent binding of a metabolite. Under the dose regimen employed in this study, atrazine does not accumulate in the rat.

Classification: Acceptable: This classification is based on the fact that the methodology requirements established in the Pesticide Assessment Guidelines, Subdivision F §85-1 have been satisfied only for reporting the distribution and excretion of atrazine in male rats. However, all of the data requirements for metabolism studies set forth in §85-1 have not been reported.

II. MATERIALS:

A. Test Compound: Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine)

Description: Not provided in this summary report.
Batch #: Not provided in this summary report.
Purity: Not provided in this summary report for the nonradiolabeled compound.
Radiolabeling procedure:
All carbons in the triazine moiety of atrazine were replaced with carbon-14. The specific activity of the radiolabeled compound was 95.8 microCuries/mg in low dose experiments and 1.06 microCuries/mg in the high dose experiments. The purity of the radiolabeled test compound was reported to be ≥ 98% ascertained by two different thin-layer chromatography systems.

B. Test Animals:

Species: Rat
Strain: Sprague-Dawley CD
Age: Not provided in this report.
Weight (mean): 160-225g
Source: Charles River Breeding Laboratories, Portage, MI (refer to p. 59 of this study).

III. STUDY DESIGN:

A. Animal Assignment:

Animals were assigned randomly to the following test groups:

Table 1
Animal Assignment in this Study
(Atrazine Elimination and Distribution Experiment)

Test Group	Daily Oral Dose Given[a] (mg/kg)	Rats male	Rats female	Duration of Exposure
1 Control	0.0	2	2	none
2 Low	1.0	5	5	1 day
3 High	100.0	5	5	1 day
4 SubQ	1.0	5	5	15 days

[a] After the last oral dose was given, the urinary and fecal levels of radioactivity were measured for 7 days.

Animals were individually placed in metabolism cages for the collection of feces and urine. The collection of metabolite was conducted at SRI International. The samples were then shipped to the CIBA-GEIGY laboratory in Greensboro, NC for analysis.

B. Dose Method: The rats were allowed a one-week acclimation period prior to initiation of experimentation. Atrazine was given orally (via a stomach tube) to the rats as an active ingredient or as a radiolabeled active ingredient. The vehicle was 3% corn starch and 0.5% polysorbate 80 (v/v). The rats were allowed free access to animal feed (Purina) and tap water.

C. Statistics:

The following procedures were utilized in analyzing the numerical data:

One- and two-way analysis of variance (ANOVA) was used to assess the statistical significance of results between dose, treatment groups or sex. When appropriate, Dunnetts or Newman-Keuls t-tests were performed to assess differences between group means.

For generating the kinetic models, the excretion data was used. This evaluation was performed by I.W.F. Davidson of Bowman Gray School of Medicine. Additional kinetic parameters such as rate constants, half-life values, and alpha and beta distribution values were obtained with the use of the ESTRIP and PCNONLIN computer programs calculated by C.M. Metzler and D.L. Weiner (Statistical Consultants, Edgewood, KY).

D. Quality Assurance:

A signed quality assurance statement was provided by a quality assurance inspector from SRI International, the subcontracting laboratory where the metabolism of radiolabeled atrazine was studied. According to the statement, the Good Laboratory Practice methods were followed in this study. However, the analytical phase of the metabolism study was reported not meet the Good Laboratory Practices Requirements of 40 CFR Part 160 because: (1) "there was no QA [quality assurance] inspection of the analytical phase of the study" and (2) " there was no QA audit of [this] final report ABR-87048."

IV. METHODS:

A. Observations: The frequency of clinical observations made on these rats was not provided in this study.

Toxicity/mortality (survival) results: There were no treatment-related deaths reported in this study.

B. Experimental Protocol: The procedure was conducted to assess the distribution (and elimination) of atrazine.

As shown in Table 1, three groups of rats (5 males and 5 females) were treated orally with atrazine. The first group received a single dose of ^{14}C-atrazine at 1 mg/kg; a second group were given a single dose of 100 mg/kg ^{14}C-atrazine; and a third group received daily doses of 1 mg/kg of nonradiolabeled atrazine for 14 days and on day 15, was given 1 mg/kg ^{14}C-atrazine. A control group received vehicle only.

Following the last dose of ^{14}C-atrazine in each group, the feces and urine were measured in each animal for 7 days. Following this, the rats were sacrificed and the urine, feces, and red blood cells, and the following selected tissues were analyzed for ^{14}C content (Figure 1).

FIGURE 1

	Digestive system
	Tongue
	Salivary glands*
	Esophagus*
	Stomach*
	Duodenum*
	Jejunum*
	Ileum*
	Cecum*
	Colon*
	Rectum*
X	Liver *+@
	Gall bladder*#
	Pancreas*
	Respiratory
	Trachea*#
X	Lung*@
	Nose^
	Pharynx^
	Larynx^

	Cardiovascular
	Aorta*
X	Heart*@.
	Bone marrow*#
	Lymph nodes*
X	Spleen@
	Thymus*
X	Red blood cell
	Urogenital
X	Kidneys*+@
	Bladder*
X	Testes*+@
	Epididymides
	Prostate
	Seminal vesicle
X	Ovaries*+@
X	Uterus*@
	Cervix
	Fallopian tubes

	Neurological
X	Brain*+@
	Peripheral nerve*#
	Spinal cord (3 levels)*#
X	Pituitary*
	Eyes (optic n.)*#
	Glandular
	Adrenal gland*
	Exorbital lacrimal gland#
X	Mammary gland*#
	Parathyroids*++
	Thyroids*++
	Other tissues
X	Bone (femur)*#
X	Muscle*#@
	Skin*#
	All gross lesions and masses*
X	Residual Carcass@
X	Fat@
X	Plasma (blood)@

* Required for subchronic and chronic studies.
^ Required for chronic inhalation.
\# In subchronic studies, examined and preserved only if indicated signs of toxicity or target organ involvement.
\+ Organ weight required in subchronic and chronic studies.
++ Organ weight required for non-rodent studies.
@ Required for determining distribution in metabolism studies.

V. **RESULTS:**

A. Distribution and Elimination of Atrazine and Its Metabolites

Five male and 5 female rats were used to assess the disposition and elimination of atrazine after acute or subchronic exposure. Table 2 shows that the total recovery of atrazine averaged 102.9% for the group given a single dose of 1 mg/kg ^{14}C-atrazine, 103.2% for the group of rats given a single dose of 100 mg/kg ^{14}C-atrazine, and 88.3% for the group of rats given a daily dose of 1 mg/kg atrazine followed by a single dose of 1 mg/kg ^{14}C-atrazine on day 15 (referred here as the subchronic group).

Concerning the elimination of atrazine or its metabolites, approximately 95% of the ^{14}C-label was excreted within 7 days of the last exposure (Table 2). In all 3 groups of rats, roughly 75% of the ^{14}C-label was excreted in the urine whereas about 20% of the ^{14}C-label was eliminated in the feces. Both discussion of other routes of elimination and the remaining 5% of the administered atrazine were not reported.

However, differences between dosage groups for tissue-borne ^{14}C-label were observed. A statistically significant decrease ($p < 0.05$) in the mean level of tissue-borne ^{14}C-label was found in those rats given a single dose of 100 mg/kg when compared to the group of rats who received a single dose of 1 mg/kg atrazine. Also, a statistically significant decrease ($p < 0.05$) in the mean level of tissue-borne ^{14}C-label was found in those rats subchronically treated with atrazine when compared to the group of rats who received a single dose of 1 mg/kg ^{14}C-atrazine. No differences were observed between sexes regarding ^{14}C-label in the urine, feces, and the tissues measured 7 days after exposure to ^{14}C-atrazine.

The red blood cells (RBC) had the highest levels of ^{14}C-label of all tissues studied (Table 3). The ratio of RBC binding of the ^{14}C-label was proportional to the dose administered, i.e., the concentration for the high dose single exposure group (100 mg/kg) was about 100 times that of the low dose single exposure group (1 mg/kg), and the tissue concentration of the subchronic group (1 mg/kg for 15 days) was the same (1.11 and 1.00) to that of the low dose group. The ratios, 1.11 and 1.00, also provide some indication that atrazine and its metabolites had not accumulated in the red blood cells or any other tissues

under this exposure regimen. This assertion is based on the observation that tissue concentrations were the same in the acute and subchronic exposure groups (Table 3).

The high concentration of ^{14}C-label reported in the red blood cell is discussed in further detail. The author suggests that ^{14}C-label binding in the red blood cell is the product of a covalent interaction between the triazine moiety of ^{14}C-atrazine and the cysteinal sulfhydryl groups in the rat hemoglobin macromolecule.

The remaining tissues listed in Table 3 show lower levels of ^{14}C-atrazine and its metabolites. Also, in these tissues, the ratio of ^{14}C-label binding was proportional lower than the administered dose, e.g., ^{14}C-label concentrations in the subchronic group were lower than that for the acute exposure group (Table 5). This finding provides evidence that atrazine or its metabolites appear not to accumulate in any tissues under this exposure regimen. However, cumulative binding of atrazine metabolites in RBCs after chronic exposure may occur.

Toxicokinetic modeling. The whole body half-life ($t_{1/2}$) of ^{14}C-label was 31.3 $\pm$ 2.8 hours (1.3 days) was calculated from the urinary excretion data. The author reported that the data best fits an open two-compartment toxicokinetic model. In addition, no statistically significant differences were reported between treatment groups or sex regarding the values for: alpha, beta, k_{10}, k_{12} and k_{21} or the whole body $t_{1/2}$ value.

Table 2
Distribution and Elimination of ^{14}C-Label (ppm) After 7 Days Following ^{14}C-Atrazine Treatment
(taken from Table I)

Dose	1.0 mg/kg		100.0 mg/kg		1.0 mg/kg (subchronic)	
Sex (#)	Males(5)	Females(5)	Males(5)	Females(5)	Males(5)	Females(5)
Urine	0.77 ±0.01	0.77 ±0.02	77.27 ±1.67	79.86 ±2.16	0.67 ±0.04	0.62 ±0.09
Feces	0.18 ±0.01	0.19 ±0.01	21.34 ±0.55	17.85 ±0.71	0.19 ±0.01	0.17 ±0.01
Tissues	0.06 ±0.001	0.07 ±0.001	4.98 ±0.13	4.48 ±0.34	0.047 ±.002	0.046 ±0.002
Cage wash[a]	0.002 ±0.0004	0.003 ±0.001	0.33 ±0.08	0.29 ±0.11	0.005 ±0.001	0.006 ±0.001
Total	1.02 ±0.01	1.04 ±0.01	103.92 ±1.44	102.48 ±2.89	0.92 ±0.44	0.85 ±0.09
% Recovery	102.9 ±1.1		103.2 ±1.5		88.3 ±4.9	

[a] At sacrifice, the cages were washed with a water acetone mixture (1:1) and 10 ml aliquots were measured for radioactivity.

Table 3
Distribution and Elimination of ^{14}C-Label (ppm) After 7 Days Following ^{14}C-Atrazine Treatment
(taken from Table IV)

Dose	1.0 mg/kg		100.0 mg/kg		1.0 mg/kg (subchronic)	
Sex (#)	Males(5)	Females(5)	Males(5)	Females(5)	Males(5)	Females(5)
RBC	0.559	0.627	67.536	62.366	0.662	0.628
Kidney	0.229	0.263	6.936	6.990	0.155	0.140
Liver	0.247	0.498	7.378	7.468	0.204	0.212
Brain	0.166	0.162	5.210	4.580	0.076	0.070
Gonads	0.147	0.198	5.124	5.799	0.066	0.050
Heart	0.144	0.154	11.726	9.770	0.137	0.102
Spleen	0.136	0.148	10.748	12.563	0.156	0.169
Lung	0.115	0.134	9.229	9.128	0.111	0.132
Pituitary	0.080	0.081	4.126	4.220	0.088	0.074
Carcass	0.076	0.080	6.349	5.901	0.069	0.061
Muscle	0.060	0.067	4.080	3.637	0.044	0.041
Bone	0.044	0.047	3.476	3.625	0.042	0.038
Fat	0.015	0.011	1.245	1.320	0.014	0.009
Plasma	0.009	0.010	1.200	1.039	0.011	0.013
Mammaries	-	0.005	-	0.346	-	0.006
Uterus	-	0.033	-	3.743	-	0.047

V. DISCUSSION:

The distribution of atrazine in rats was found to be dose-dependent and independent of sex. Of the tissues studied, the red cells store the highest levels of atrazine, apparently through the covalent binding of a metabolite. In rats given a single oral dose of 100 mg/kg atrazine, in decreasing order, the levels found in the following tissues were: red blood cells, heart, spleen, lung, liver, kidney, brain, gonads, pituitary, muscle, bone, fat, and plasma. In rats given repeated daily oral doses of 1 mg/kg atrazine, in decreasing order, the levels found in the following tissues were: red blood cells, liver, spleen, kidney, lung, heart, pituitary, brain, gonads, muscle, bone, fat, and plasma. Under the exposure regimen used in this study, atrazine does not accumulate in the tissues of the rat, except perhaps for the red blood cell. The pattern of atrazine tissue distribution for atrazine in this study is similar to that found in rats repeated exposed to atrazine (MRID Nos. 404313-05 and 404313-09).

The distribution of atrazine was reported to follow first-order kinetics. Two major relationships are found: (1) the whole body half-life, or $t_{1/2}$, and the volume of distribution, or V_d, are independent of the dose of atrazine and (2) the plasma concentration of atrazine or its metabolites are directly proportional to the dose of atrazine. Plasma concentrations of atrazine measured during and after atrazine exposure showed that the whole body half-life ($t_{1/2}$) of atrazine or its metabolites is 31.3 hours (1.3 days) in rats. These reported findings further indicate that atrazine does not accumulate under this exposure regimen in the rat.

Excretion of atrazine in rats. About 95% of the atrazine administered orally is excreted within 7 days after cessation of exposure. The route of atrazine excretion was reported to be independent of the dose and sex of rat. About 75% of the atrazine is excreted through the urinary route whereas about 20% of the atrazine is eliminated in the feces. The elimination route for the remaining 5% was not reported. Also, the level of atrazine elimination by exhalation or through the skin (sweating) was not reported.

Summary. The whole body half-life of 1.30 days for atrazine is consistent with the observation that about 95% of the administered dose is eliminated within 7 days after exposure. The red cells store the highest concentration of atrazine in the rat, apparently through the covalent binding of a metabolite. Under the dose regimen employed in this study, atrazine does not accumulate in the rat.

Classification: Acceptable: This classification is based on the fact that the methodology requirements established in the Pesticide Assessment Guidelines, Subdivision F §85-1 have been satisfied only for reporting the distribution and excretion of atrazine in male rats. However, all of the data requirements for metabolism studies set forth in §85-1 have not been reported.

Reviewed by: Sanford W. Bigelow, Ph.D. 5/6/88
Section VI, Toxicology Branch (TS-769C)
Secondary reviewer: Judith W. Hauswirth, Ph.D. Judith W. Hauswirth 5/9/88
Section VI, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

I. SUMMARY:

STUDY TYPE: Metabolism - rat (85-1) CASWELL NO: 63

ACCESSION NUMBER: MRID NO.: 404375-01

TEST MATERIAL: Atrazine

SYNONYMS: 2-Chloro-4-ethylamino-6-isopropylamino-s-triazine

STUDY NUMBER: ABR-87116

SPONSOR: CIBA-GEIGY Corp., Agricultural Division, P.O. Box 18300 Greensboro, NC 27419 Thomas Parshley, Regulatory Specialist (919) 292-7100 X7207

TESTING FACILITY: CIBA-GEIGY Corp., Biochemistry Dept., P.O. Box 18300 Greensboro, NC 27419

TITLE OF REPORT: A Summary of the Disposition, Kinetics and Metabolism of Atrazine in the Rat (General Metabolism).

AUTHOR: G.R. Orr

REPORT ISSUED: November 17, 1987

CONCLUSIONS:

The summary data regarding the distribution, metabolism and the elimination of atrazine were provided in this report. To this end, three separate experiments were conducted with the use of three groups of rats. Radiolabeled atrazine (triazine ring, uniformly labeled) was used by the author to measure the disposition of atrazine and/or its metabolites in the rat. The first experiment was performed to assess the distribution and elimination of atrazine in male and female rats repeatedly exposed to daily doses of atrazine. The second experiment was performed to assess in further detail the distribution of atrazine in female rats, especially in the red blood cell. The third experiment was conducted to identify the urinary metabolites of atrazine formed by the female rat. The absorption of atrazine in male or female rats was not reported.

Absorption of atrazine in rats. No data was provided in this summary report regarding the absorption of atrazine in rats.

Distribution of Atrazine in rats. The distribution of atrazine in rats was found to be dose-dependent and independent of sex. Of the tissues studied, the red blood cells store the highest levels of atrazine, apparently through the covalent binding of a metabolite. In rats given a single dose of 100 mg/kg atrazine, in decreasing order, the levels found in the following tissues were: heart, spleen, lung, liver, kidney, brain, gonads, pituitary, muscle, uterus, bone, fat, and plasma. Under the exposure regimen used in this study, atrazine does not accumulate in the tissues of the rat, except perhaps for the red blood cell.

The pattern of tissue distribution of atrazine in rats repeatedly exposed differs from that of rats given a single exposure of atrazine. In rats given repeated daily oral doses of 1 mg/kg atrazine, in decreasing order, the levels found in the following tissues were: red blood cells, liver, spleen, kidney, lung, heart, pituitary, brain, gonads, muscle, bone, fat, and plasma. Under the exposure regimen used in this study, atrazine does not accumulate in the tissues of the rat, except perhaps for the red blood cell. The pattern of atrazine tissue distribution for atrazine in this study is similar to that found in rats repeated exposed to atrazine (see MRID Nos. 404313-04, 404313-05 and 404313-09 for more detail).

The distribution of atrazine was reported to follow first-order kinetics. Two major relationships are found: (1) the whole body half-life, or $t_{1/2}$, and the volume of distribution, or V_d, are independent of the dose of atrazine and (2) the plasma concentration of atrazine or its metabolites are directly proportional to the dose of atrazine. Plasma concentrations of atrazine measured during and after atrazine exposure showed that the whole body half-life ($t_{1/2}$) of atrazine or its metabolites is 38.6 hours (1.61 days) in rats. These reported findings further indicate that atrazine does not accumulate under this exposure regimen in the rat.

As mentioned above, the highest level of atrazine was found in the RBC. The estimated half-life of 8.14 days in RBCs (as compared to the whole body half-life of 1.61 days) indicates that extensive binding of atrazine or its metabolites in RBCs was occurring. However, after cessation of multiple exposure, the concentration of atrazine or its metabolites in RBCs declined at all doses except for the highest dose, 100 mg/kg atrazine.

Excretion of atrazine in rats. About 95% of the atrazine administered orally is excreted within 7 days after cessation of exposure. The route of atrazine excretion was reported to be independent of the dose and sex of rat. About 75% of the atrazine is excreted through the urinary route whereas about 20% of the atrazine is eliminated in the feces. The elimination route for the remaining 5% was not reported. Also, the level of atrazine elimination by exhalation or through the skin (sweating) was not reported.

Metabolism of atrazine in rats. The data reported in this study indicates that dechlorination of the triazine ring and N-dealkylation are the major metabolic pathways for atrazine in rats. Oxidation of the alkyl substituents of atrazine appears to be a minor and secondary metabolic route.

The author argues that a "carbon-sulfur lyase," cleaves the glutathione residue from an atrazine metabolite to produce a thiol-containing atrazine metabolite. The author further posits that the action of the lyase results in the covalent binding of the thiol-containing atrazine metabolite to hemoglobin in the red blood cell, a finding from the multiple exposure studies (depicted in Table 7). However, the author has not provided evidence in this study whether lyase is present in red blood cells.

Summary. The whole body half-life of 1.61 days for atrazine is consistent with the observation that 95% of the administered dose is elimination within 7 days after exposure. The red cells store the highest concentration of atrazine in the rat, apparently through the covalent binding of a metabolite. Under the dose regimen employed in this study, atrazine does not accumulate in the rat.

Classification: Acceptable: This classification is based on the fact that the methodology requirements established in the Pesticide Assessment Guidelines, Subdivision F §85-1 have been satisfied only for reporting (1) the identity of urinary metabolites of atrazine in female rats as well as (2) the distribution and excretion of atrazine in male and female rats. However, all of the data requirements for metabolism studies set forth in Subdivision F §85-1 have not been reported, i.e., (a) the urinary and fecal metabolites of atrazine in male rats and (b) the fecal metabolites of atrazine in females must be identified to completely satisfy the §85-1 data reporting requirements for the metabolism of atrazine in the rat.

006718

II. MATERIALS:

A. Test Compound: Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine)

Description: Not provided in this summary report.
Batch #: Not provided in this summary report.
Purity: Not provided in this summary report.
Radiolabeling procedure:
All carbons in the triazine moiety of atrazine were replaced with carbon-14. [The specific activity of the radiolabeled compound was not reported in this summary report as required by FIFRA Subdivision F test guidelines §85-1 (b)(2)(i).]

B. Test Animals:

Species: Rat (female and male)
Strain: Sprague-Dawley CD
Age: Not provided in this summary report.
Weight (mean): about 0.2 kg (reported only for Experiment #3)
Source: Charles River

III. STUDY DESIGN:

A. Animal Assignment:

Animals were assigned randomly to the following test groups:

Table 1
Animal Assignment in this Study
(Atrazine Elimination and Distribution Experiment)

Test Group	Daily Oral Dose Given[a] (mg/kg)	Rats male	Rats female	Duration of Exposure
1 Low	1.0	5	5	1 day
2 High	100.0	5	5	1 day
3 SubQ	1.0	5	5	15 days

[a] After the last oral dose was given, the urinary and fecal levels of radioactivity were measured for 7 days.

Table 2
Animal Assignment in this Study
(Atrazine Distribution Experiment)

Test Group	Daily Oral Dose Given (mg/kg)	Rats (female)	Duration of Exposure (days)
1 Control	0	2	10
2 Low1 (LDT1)	1.0	2	10
3 Low2 (LDT2)	3.0	2	10
4 Low3 (LDT3)	7.0	2	10
5 Low4 (LDT4)	10.0	2	10
6 Mid1 (MDT1)	50.0	2	10
7 High (HDT1)	100.0	2	10

Table 3
Animal Assignment in this Study
(Atrazine Metabolism Experiment)

Test Group	Daily Oral Dose Given (mg/kg)	Rats (female)	Duration of Exposure (day)
1 High	100.0	5	1
2 Mid	16.2 - 19.6	8	1

B. Diet Preparation: Atrazine was was given orally to the rats (via a stomach tube) as an active ingredient or as a radiolabeled active ingredient. Animals were allowed free access to animal feed (Purina) and tap water. The animals were allowed a one-week acclimation period prior to initiation of experimentation.

C. Statistics:

The following procedure was utilized in analyzing the numerical data:

Analysis of variance (ANOVA) was used to assess the statistical significance of results between treatment groups and sexes.

D. Quality Assurance:

A signed quality assurance statement was provided by a quality assurance inspector from SRI International, the subcontracting laboratory where the metabolism of radiolabeled atrazine was studied. According to the statement, the Good Laboratory Practice methods were followed in this study. However, the analytical phase of the metabolism study (as stated in Study No. ABR-87048) was reported not meet the Good Laboratory Practices Requirements of 40 CFR Part 160 because: (1) "there was no QA [quality assurance] inspection of the analytical phase of the study" and (2) " there was no QA audit of [this] final report ABR-87048."

IV. METHODS:

A. Observations: The frequency of clinical observations made on these rats was not provided in this summary report.

Toxicity/mortality (survival) results: There were no treatment-related deaths reported in this study.

B. Atrazine dosage regimens: Three separate experiments were conducted with the use of three groups of rats. The first experiment was performed to assess the distribution and elimination of atrazine. The second experiment was performed to assess in further detail the distribution of atrazine, especially in the red blood cell. The third experiment was conducted to identify the atrazine metabolites formed by the rat.

1. Experiment #1. As shown in Tables 1, 2, and 3, respectively, three groups of rats (5 males and 5 females) were treated orally with atrazine. The first group received a single oral dose of ^{14}C-atrazine at 1 mg/kg; a second group were given a single oral dose of 100 mg/kg ^{14}C-atrazine; and a third group received daily oral doses of 1 mg/kg of nonradiolabeled atrazine for 14 days and on day 15, was given 1 mg/kg ^{14}C-atrazine.

Following the last dose of ^{14}C-atrazine in each group, the feces and urine were collected in each animal for 7 days. Following this, the rats were sacrificed and the urine, feces, and red blood cells, and the following selected tissues were analyzed for ^{14}C content (Figure 1).

FIGURE 1

Digestive system		**Cardiovascular**		**Neurological**	
	Tongue		Aorta*	X	Brain*+@
	Salivary glands*	X	Heart*@		Peripheral nerve*#
	Esophagus*		Bone marrow*#		Spinal cord (3 levels)*#
	Stomach*		Lymph nodes*	X	Pituitary*
	Duodenum*	X	Spleen@		Eyes (optic n.)*#
	Jejunum*		Thymus*		
		X	Red blood cell		**Glandular**
	Ileum*	**Urogenital**			Adrenal gland*
	Cecum*	X	Kidneys*+@		Exorbital lacrimal gland#
	Colon*		Bladder*	X	Mammary gland*#
	Rectum*	X	Testes*+@		Parathyroids*++
X	Liver *+@		Epididymides		Thyroids*++
	Gall bladder*#		Prostate	**Other tissues**	
	Pancreas*		Seminal vesicle	X	Bone (femur)*#
Respiratory		X	Ovaries*+@	X	Muscle*#@
	Trachea*#	X	Uterus*@		Skin*#
X	Lung*@		Cervix		All gross lesions and masses*
	Nose^		Fallopian tubes	X	Residual Carcass@
	Pharynx^			X	Fat@
	Larynx^			X	Plasma (blood)@

* Required for subchronic and chronic studies.
^ Required for chronic inhalation.
\# In subchronic studies, examined and preserved only if indicated signs of toxicity or target organ involvement.
\+ Organ weight required in subchronic and chronic studies.
++ Organ weight required for non-rodent studies.
@ Required for determining distribution in metabolism studies.

2. Experiment #2. As listed in Table 2, in an effort to study in more detail the toxicokinetic disposition of ^{14}C-atrazine as a function of the dose of atrazine and the time of sacrifice, six groups of female Sprague-Dawley rats were treated with ^{14}C-atrazine while another group of female rats served as a control group. The groups of rats were given a daily oral dose for 10 consecutive days at 0 mg/kg (vehicle only), 1 mg/kg, 3 mg/kg, 7 mg/kg, 10 mg/kg, 50 mg/kg, and 100 mg/kg ^{14}C-atrazine. The vehicle was an aqueous solution of corn starch/polysorbate-80. Urine and feces were collected daily. At 24, 48, 72, 96, 144, 192, 219, 240, 264 and 288 hours, blood samples were obtained via orbital puncture. Five milliliters of blood were collected by aortal puncture at sacrifice. The tissues selected for determining the distribution of ^{14}C-label at each dose are listed in Figure 2. One of the two animals

in each group was sacrificed 3 hours after the tenth dose of ^{14}C-atrazine and the other animal in each group was sacrificed 72 hours after the tenth dose of ^{14}C-atrazine. The distribution of ^{14}C-label in the urine, feces, red blood cells, and the following selected tissues was determined for each female rat (Figure 2).

FIGURE 2

Digestive system		**Cardiovascular**		**Neurological**	
	Tongue		Aorta*	X	Brain*$_{+}$@
	Salivary glands*		Heart*@		Peripheral nerve*#
	Esophagus*		Bone marrow*#		Spinal cord (3 levels)*#
	Stomach*		Lymph nodes*	X	Pituitary*
	Duodenum*		Spleen@		Eyes (optic n.)*#
	Jejunum*		Thymus*		
		X	Red blood cell		**Glandular**
	Ileum*	**Urogenital**			Adrenal gland*
	Cecum*	X	Kidneys*+@		Exorbital lacrimal gland#
	Colon*		Bladder*	X	Mammary gland*#
	Rectum*		Testes*$^{+}$@		Parathyroids*$^{++}$
X	Liver *$^{+}$@		Epididymides		Thyroids*$^{++}$
	Gall bladder*#		Prostate	**Other tissues**	
	Pancreas*		Seminal vesicle		Bone (femur)*#
Respiratory		X	Ovaries*$^{+}$@		Muscle*#@
	Trachea*#		Uterus*@		Skin*#
	Lung*@		Cervix		All gross lesions and masses*
	Nose^		Fallopian tubes		Residual Carcass@
	Pharynx^				Fat@
	Larynx^				Plasma (blood)@

* Required for subchronic and chronic studies.
^ Required for chronic inhalation.
\# In subchronic studies, examined and preserved only if indicated signs of toxicity or target organ involvement.
\+ Organ weight required in subchronic and chronic studies.
++ Organ weight required for non-rodent studies.
@ Required for determining distribution in metabolism studies.

3. Experiment #3. As shown in Table 3, one group of female rats was given a single oral dose of atrazine in an effort to produce sufficient levels of urinary metabolites of atrazine for identification. Five adult female Sprague-Dawley rats (about 0.2 kg) were administered 100 mg/kg ^{14}C-atrazine. Samples of urine and feces were obtained at 24, 48, and 72 hours. After taking samples for 72 hours, the rats were sacrificed and 5 ml of blood and the liver were obtained.

In another exposure trial, 8 rats were given a single oral exposure of 16.18 - 19.64 mg/kg ^{14}C-atrazine. Urinary metabolites were collected over a 24-hour period following treatment. From this exposure, atrazine metabolites were positively identified by confirmation with infrared and mass spectra.

V. RESULTS:

A. Distribution and Elimination of Atrazine and Its Metabolites

Experiment #1. The 5 male and 5 female rats were used to assess the disposition and elimination of atrazine after single or multiple oral doses of atrazine. Table 4 shows that the total recovery of atrazine averaged 102.9% for the group given a single dose of 1 mg/kg ^{14}C-atrazine, 103.2% for the group of rats given a single dose of 100 mg/kg ^{14}C-atrazine, and 88.3% for the group of rats given a daily dose of 1 mg/kg atrazine followed by a single dose of 1 mg/kg ^{14}C-atrazine on day 15 (referred here as the multiple dosing or the multiple exposure group).

Concerning the elimination of atrazine or its metabolites, approximately 95% of the ^{14}C-label was excreted within 7 days of the last exposure (Table 4). In all 3 groups of rats, roughly 75% of the ^{14}C-label was excreted in the urine whereas about 20% of the ^{14}C-label was eliminated in the feces. Both discussion of other routes of elimination and the remaining 5% of the administered atrazine were not reported.

However, differences between dosage groups for tissue-borne ^{14}C-label were observed. A statistically significant decrease ($p < 0.05$) in the mean level of tissue-borne ^{14}C-label was found in those rats given a single dose of 100 mg/kg when compared to the group of rats who received a single dose of 1 mg/kg atrazine. Also, a statistically significant decrease ($p < 0.05$) in the mean level of tissue-borne ^{14}C-label was found in those rats treated with multiple oral doses of atrazine when compared to the group of rats who received a single oral dose of 1 mg/kg ^{14}C-atrazine. No differences were observed between sexes regarding the percentage of ^{14}C-label that was excreted in the urine and feces (Table 4). The pattern for tissue distributed between single and multiple exposure groups were similar (Table 5) collected 7 days after exposure to ^{14}C-atrazine.

The red blood cells (RBC) had the highest levels of ^{14}C-label of all tissues studied (Table 5). The ratio of RBC binding of the ^{14}C-label was proportional to the dose administered, i.e., the concentration for the high dose single exposure group (100 mg/kg) was about 100 times that of the low dose single exposure group (1 mg/kg), and the tissue concentration of the multiple dose group (1 mg/kg for 15 days) was the same (1.11 and 1.00) to that of the

low dose group. The ratios, 1.11 and 1.00, also provide some indication that atrazine and its metabolites had not accumulated in the red blood cells or any other tissues under this exposure regimen. This assertion is based on the observation that tissue concentrations were the same in single and multiple oral exposure groups (Table 5).

Moreover, the authors suggest that ^{14}C-label binding in the red blood cell is the product of a covalent interaction between the triazine moiety of ^{14}C-atrazine and the cysteinal sulfhydryl groups in the rat hemoglobin macromolecule. This binding is discussed later in more detail in the metabolism section of this review.

The remaining tissues listed in Table 5 show lower levels of ^{14}C-atrazine and its metabolites. Also, in these tissues, the ratio of ^{14}C-label binding was proportionally lower than the administered dose, e.g., ^{14}C-label concentrations in the multiple expsoure group were lower than that for the single exposure group (Table 5). This finding provides evidence that atrazine or its metabolites appear not to accumulate in any tissues under this exposure regimen. However, cumulative binding of atrazine metabolites in RBCs after chronic exposure may occur.

Toxicokinetic modeling. The data generated from urinary excretion were used to calculate the:

- half-life values ($t_{1/2}$) of the alpha distributive phase, and
- the whole body half-life value of the beta excretory phase.

The author reported that the data best fit an open two-compartment toxicokinetic model. No statistically significant differences were reported between treatment groups or sex regarding the values for: alpha, beta, k_{10}, k_{12} and k_{21} or any $t_{1/2}$ value.

Table 4
Distribution and Elimination of ^{14}C-Label (ppm) After 7 Days Following ^{14}C-Atrazine Treatment
(taken from Table I)

Dose	1.0 mg/kg		100.0 mg/kg		1.0 mg/kg (multiple doses)	
Sex (#)	Males(5)	Females(5)	Males(5)	Females(5)	Males(5)	Females(5)
Urine	0.77 ±0.01	0.77 ±0.02	77.27 ±1.67	79.86 ±2.16	0.67 ±0.04	0.62 ±0.09
Feces	0.18 ±0.01	0.19 ±0.01	21.34 ±0.55	17.85 ±0.71	0.19 ±0.01	0.17 ±0.01
Tissues	0.06 ±0.001	0.07 ±0.001	4.98 ±0.13	4.48 ±0.34	0.047 ±.002	0.046 ±0.002
Cage wash	0.002 ±0.0004	0.003 ±0.001	0.33 ±0.08	0.29 ±0.11	0.005 ±0.001	0.006 ±0.001
Total	1.02 ±0.01	1.04 ±0.01	103.92 ±1.44	102.48 ±2.89	0.92 ±0.44	0.85 ±0.09
% Recovery	102.9 ±1.1		103.2 ±1.5		88.3 ±4.9	

Table 5
Distribution and Elimination of ^{14}C-Label (ppm) After 7 Days Following ^{14}C-Atrazine Treatment (taken from Table IV)

Dose	1.0 mg/kg		100.0 mg/kg		1.0 mg/kg (multiple doses)	
Sex (#)	Males(5)	Females(5)	Males(5)	Females(5)	Males(5)	Females(5)
RBC	0.559	0.627	67.536	62.366	0.662	0.628
Kidney	0.229	0.263	6.936	6.990	0.155	0.140
Liver	0.247	0.498	7.378	7.468	0.204	0.212
Brain	0.166	0.162	5.210	4.580	0.076	0.070
Gonads	0.147	0.198	5.124	5.799	0.066	0.050
Heart	0.144	0.154	11.726	9.770	0.137	0.102
Spleen	0.136	0.148	10.748	12.563	0.156	0.169
Lung	0.115	0.134	9.229	9.128	0.111	0.132
Pituitary	0.080	0.081	4.126	4.220	0.088	0.074
Carcass	0.076	0.080	6.349	5.901	0.069	0.061
Muscle	0.060	0.067	4.080	3.637	0.044	0.041
Bone	0.044	0.047	3.476	3.625	0.042	0.038
Fat	0.015	0.011	1.245	1.320	0.014	0.009
Plasma	0.009	0.010	1.200	1.039	0.011	0.013
Mammaries	-	0.005	-	0.346	-	0.006
Uterus	-	0.033	-	3.743	-	0.047

B. Distribution of radiolabeled atrazine after repeated daily dosing and multiple sampling.

Another experiment was conducted with a protocol designed to determine the bodily disposition of ^{14}C-label after multiple doses of ^{14}C-atrazine. The recovery of the total dose averaged 89.2% in rats killed 3 hours after the tenth dose of ^{14}C-atrazine and 94.2% in rats killed 72 hours after the tenth dose of ^{14}C-atrazine averaged. The amount of ^{14}C-label of the total dose excreted in the feces in rats killed at 3 hours was 13.4% and was 14.8% in rats killed at 72 hours independent of the dose. The amount of ^{14}C-label of the total dose excreted in the urine was 69.5% in the rats killed at 3 hours and 76.3% in the rats killed at 72 hours independent of the dose. The total percentage of the initial dose ^{14}C-atrazine excreted in the urine and feces in the rats killed at 3 hours was 82.9% and in the rats killed at 72 hours was 91.1%.

Plasma concentrations of atrazine. In this multiple dosing experiment, plasma concentrations were related linearly to the dose of ^{14}C-atrazine (Table 6). That is, plasma concentrations in rats given 100 mg/kg ^{14}C-atrazine were roughly 100 times that of rats given 1 mg/kg ^{14}C-atrazine. This comparison applies to all of the dosage groups at most time points listed in Table 6. Overall, during daily dosing plasma levels of atrazine or its metabolites generally rose and reached an apparent plateau or steady-state. After daily dosing had stopped the following toxicokinetic values were calculated from the data obtained:

- the whole body half-life, or $t_{1/2}$, of 38.6 hours (1.61 days) for the elimination of atrazine or its metabolites,
- the estimated volume of distribution, or V_d, for the daily dose of 10 mg/kg was 4.15 L/kg, and
- at a dose of 10 mg/kg, the mean plasma concentration of atrazine or its metabolites at steady-state was 5.61 mg-equivalents ^{14}C-label/L of plasma.

For distribution models that follow first-order kinetics such as this model proposed for atrazine, two relationships are found: (1) $t_{1/2}$ and V_d are independent of the dose and (2) the plasma concentration of ^{14}C-label is directly proportional to the dose of ^{14}C-atrazine.

Table 6
Plasma Levels of ^{14}C-Label (ppm) During the Dosing Period and at Sacrifice
(taken from Table VIII)

Dose	1 mg/kg		3 mg/kg		7 mg/kg		10 mg/kg		50 mg/kg		100 mg/kg	
Rat #:	R5062	R5063	R5064	R5065	R5066	R5067	R5068	R5069	R5070	R5071	R5072	R5073
Hour of Sacrifice:	3	72	72	3	3	72	3	72	3	72	3	72
Time (hrs.):												
24	0.068	0.061	0.053	0.375	0.741	0.562	1.062	1.164	9.291	8.279	27.104	22.298
48	0.186	0.181	0.452	0.615	1.058	1.884	2.009	1.593	7.161	.129?	28.946	23.101
72	0.469	0.405	1.383	1.468	3.267	3.248	4.168	3.957	20.911	17.778	40.320	57.877
96	0.506	0.596	1.808	1.845	3.311	3.747	4.165	4.661	21.249	24.448	52.271	56.580
144	0.582	0.594	2.150	2.608	4.225	3.359	5.069	5.109	25.169	24.604	59.751	69.514
192	0.560	0.658	1.668	1.941	4.066	3.533	5.343	4.725	23.437	27.682	48.671	44.420
219	0.583	--	--	1.406	3.748	--	5.067	--	21.351	--	51.715	--
240	--	0.185	0.703	--	--	1.628	--	3.099	--	26.413	--	29.566
264	--	0.144	0.789	--	--	1.371	--	1.713	--	13.352	--	17.682
288	--	0.117	0.340	--	--	0.868	--	1.600	--	8.302	--	14.775

RBC concentrations of atrazine. The same experimental method used for determining plasma concentrations of atrazine and its metabolites was employed to measure the level of ^{14}C-label in red blood cells (RBCs). The concentration of ^{14}C-label in RBCs rose during repeated daily dosing of ^{14}C-atrazine and did not reach a plateau or steady state (Table 7). RBC concentrations appeared to be proportional (usually supralinear) to the dose of ^{14}C-atrazine. After cessation of daily dosing, the concentration of ^{14}C-label declined for all doses except the highest dose, 100 mg/kg ^{14}C-atrazine.

After daily dosing was stopped, the data was obtained from the level of ^{14}C-label in the urine. The following toxicokinetic values were calculated from those data:

- the mean dosage half-life, or $t_{1/2}$, was 1562.9 hours (8.14 days) for the elimination of atrazine or its metabolites from RBCs,
- the estimated volume of distribution, or V_d, for the daily dose of 10 mg/kg was 0.7 L/kg, and
- at a dose of 10 mg/kg, the mean plasma concentration of atrazine or its metabolites at steady-state was 104.6 mg-equivalents ^{14}C-label/L of cells.

The RBC:plasma concentration ratio was roughly related linearly in all dose levels. The estimated half-life of 8.14 days and the large volume of distribution 104.6 mg-equivalents/L) in RBCs indicate that extensive binding of atrazine and its metabolites in RBCs were occurring. (The life span of a rat RBC is 45-56 days). The authors speculate that binding of ^{14}C-label is of a covalent nature.

Table 7
Red Blood Cell Levels of ^{14}C-Label (ppm) During the Dosing Period and at Sacrifice
(taken from Table IX)

Dose	1 mg/kg		3 mg/kg		7 mg/kg		10 mg/kg		50 mg/kg		100 mg/kg	
Rat #:	R5062	R5063	R5064	R5065	R5066	R5067	R5068	R5069	R5070	R5071	R5072	R5073
Hour of Sacrifice:	3	72	72	3	3	72	3	72	3	72	3	72
Time (hrs.):												
24	0.93	0.54	2.57	4.67	5.19	6.39	7.31	7.81	50.87	36.59	109.06	70.38
48	1.48	1.27	4.29	8.48	12.82	14.17	20.72	15.88	124.35	87.00	234.05	190.48
72	2.64	2.77	--	--	19.98	23.08	31.55	26.71	177.56	129.41	292.78	275.59
96	3.51	2.76	21.47	22.01	26.65	30.38	39.07	37.59	225.49	160.46	474.56	305.37
144	5.18	4.24	--	--	37.07	30.78	60.65	53.79	358.75	289.12	881.41	649.45
192	6.61	5.04	23.44	11.63	43.78	50.05	63.84	63.85	415.80	362.30	695.14	529.04
219	10.03	--	--	21.20	54.98	--	83.92	--	307.74	--	517.23	--
240	--	5.98	18.57	--	--	51.45	--	85.63	--	324.18	--	551.40
264	--	5.41	18.11	--	--	46.65	--	67.83	--	318.95	--	605.28
288	--	5.34	18.50	--	--	50.77	--	41.26	--	271.48	--	611.42

Tissue concentrations of atrazine. The tissue concentrations of atrazine and its metabolites were measured in selected tissues from animals killed at 3 and at 72 hours (Table 8). At all doses, tissue levels of ^{14}C-label are consistently lower in all animals killed 72 hours after cessation of ^{14}C-atrazine exposure, a finding that corroborates the observed decline in plasma concentration of ^{14}C-label (Table 6). The liver had the highest tissue concentration of ^{14}C-label, followed by the kidney, pituitary and ovary. The brain had the lowest tissue concentration in this experiment. In respect to making dose comparisons, tissue levels of ^{14}C-label were generally supralinear, i.e., the tissue level in rats given 100 mg/kg ^{14}C-atrazine was generally 200 times higher than that of rats given 1 mg/kg ^{14}C-atrazine. In animals sacrificed at 72 hours, the mammary tissue:plasma concentration ratio at 1 mg/kg was 0.042 and at 100 mg/kg was 0.49; a difference that is roughly proportional to the dose of atrazine.

Table 8
Tissue Levels of ^{14}C-Label (ppm) at Sacrifice
(taken from Table X)

Dose	1 mg/kg		3 mg/kg		7 mg/kg		10 mg/kg		50 mg/kg		100 mg/kg	
Rat #:	R5062	R5063	R5064	R5065	R5066	R5067	R5068	R5069	R5070	R5071	R5072	R5073
Hour of Sacrifice:	3	72	72	3	3	72	3	72	3	72	3	72
Tissue:												
Liver	2.97	2.33	5.40	8.06	16.21	8.05	20.86	12.37	54.87	32.58	102.37	55.88
Pituitary	1.18	0.50	1.67	3.54	7.32	3.24	9.36	4.49	36.91	18.18	71.68	33.90
Ovary	1.14	0.48	1.59	3.62	7.28	2.81	9.08	4.69	36.30	17.42	76.39	33.02
Brain	0.39	0.24	0.90	1.57	3.24	1.55	4.12	2.04	14.59	8.99	30.25	11.84
Kidney	1.36	0.61	1.64	3.54	6.91	3.35	9.88	4.70	29.31	16.73	78.64	26.13
Mammaries:												
Pectoral	0.13	0.05	0.38	0.46	0.52	0.24	1.24	0.75	4.41	3.32	6.30	7.33
Inguinal	0.06	0.05	0.15	0.19	0.79	0.11	0.54	0.65	4.06	2.29	4.77	0.33

B. The Metabolism of Atrazine

To examine the metabolism of atrazine in rats, 100 mg/kg of ^{14}C-atrazine was given to rats and the ^{14}C-labeled metabolites were isolated and identified. A recovery of 103.78% of the total radioactivity was achieved. The urinary route accounted for 47.4% of the elimination whereas 49.3% of the ^{14}C-label was eliminated via the fecal route. The tissues contained 5.75% of the ^{14}C-label while the blood contained the remaining 1.4% of the ^{14}C-label.

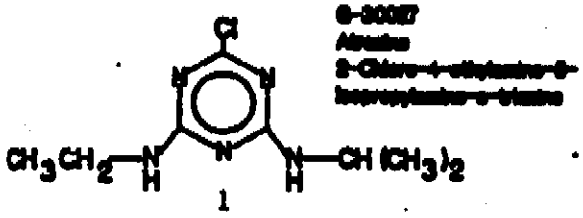
In vivo metabolism of atrazine. The molecular structures of the urinary metabolites obtained from the first group rats were unattainable, so a second group of 8 rats were given 16.18-19.64 mg/kg ^{14}C-atrazine. The metabolites were collected within the 0 to 24 hour time period after exposure. The urine was freeze dried. Then the metabolites were dissolved in a small amount of water that was acidified with HCl to pH 3.0 and separated with an amino acid analyzer (to detect the amino acid residues of glutathione) coupled with a cation exchange column.

A total of 19 radioactive peaks were detected, three of which were identified as metabolites by comparison of the infrared and mass spectra. The identity of two other metabolites was postulated based on additional mass spectral information. The molecular structures of some of the atrazine metabolites are shown in Figure 1 and the numbers in this figure correspond to the metabolites discussed in the text. Eight metabolites were identified and the major metabolites are listed below:

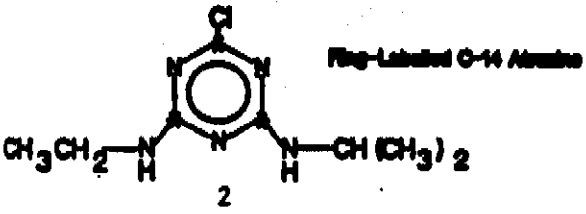
- 2-hydroxy-atrazine (7),
- 2-hydroxy-4-amino-6-isopropylamino-s-triazine (8),
- 2-hydroxy-4-ethylamino-6-amino-s-triazine (14), and
- 2-hydroxy-4,6-diamino-s-triazine (3).

The identification of the major metabolites above indicates that dechlorination of the triazine ring and N-dealkylation are the major metabolic pathways for atrazine in rats. Because four other minor metabolites that possess omega-carboxyl moieties were identified (5, 10, 11, 12), oxidation of the terminal methyl moieties in the alkyl substituents appears to be a minor and secondary metabolic route.

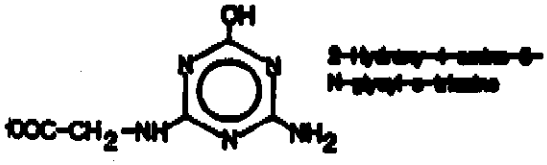
FIGURE I. CHEMICAL NAMES AND STRUCTURES



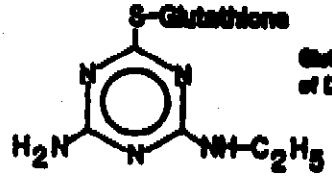
1



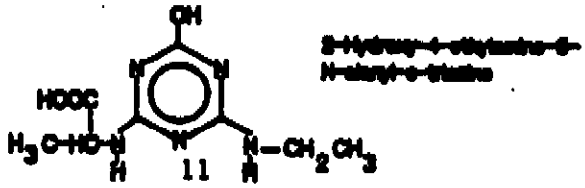
2



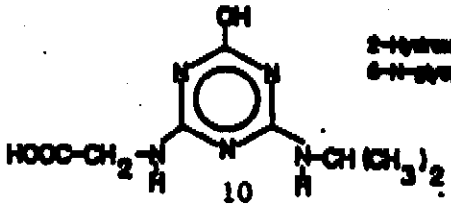
5



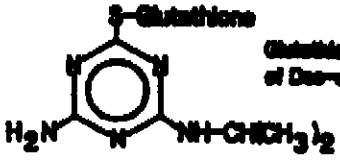
18



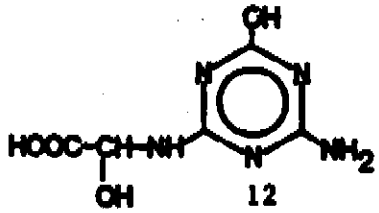
11



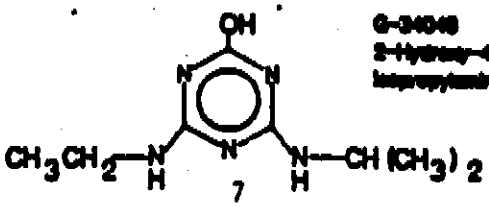
10



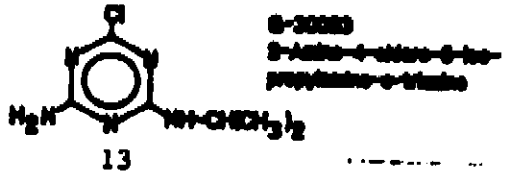
17



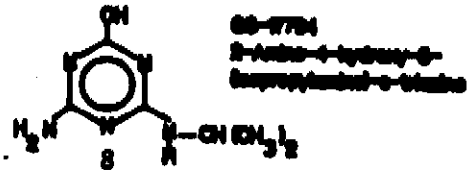
12



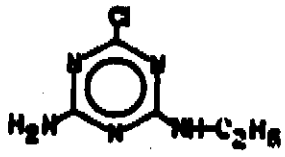
7



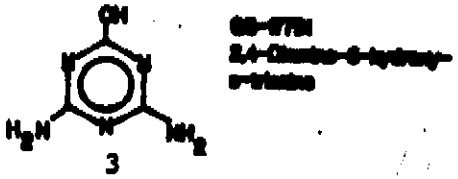
13



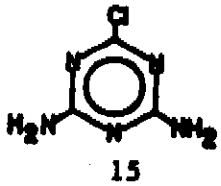
8



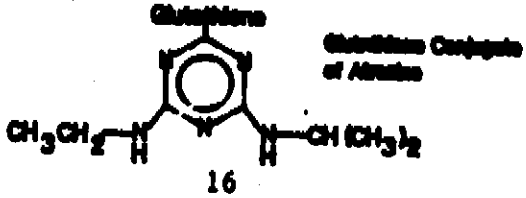
14



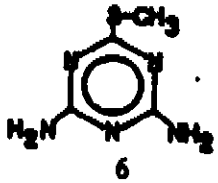
3



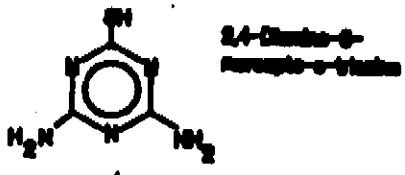
15



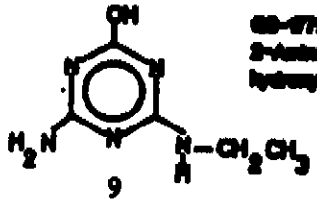
16



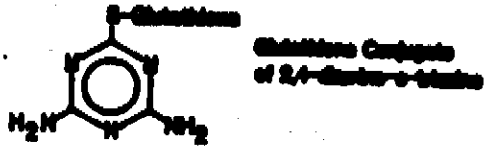
6



4



9



19

158

FIGURE I. CHEMICAL NAMES AND STRUCTURES (Cont.)

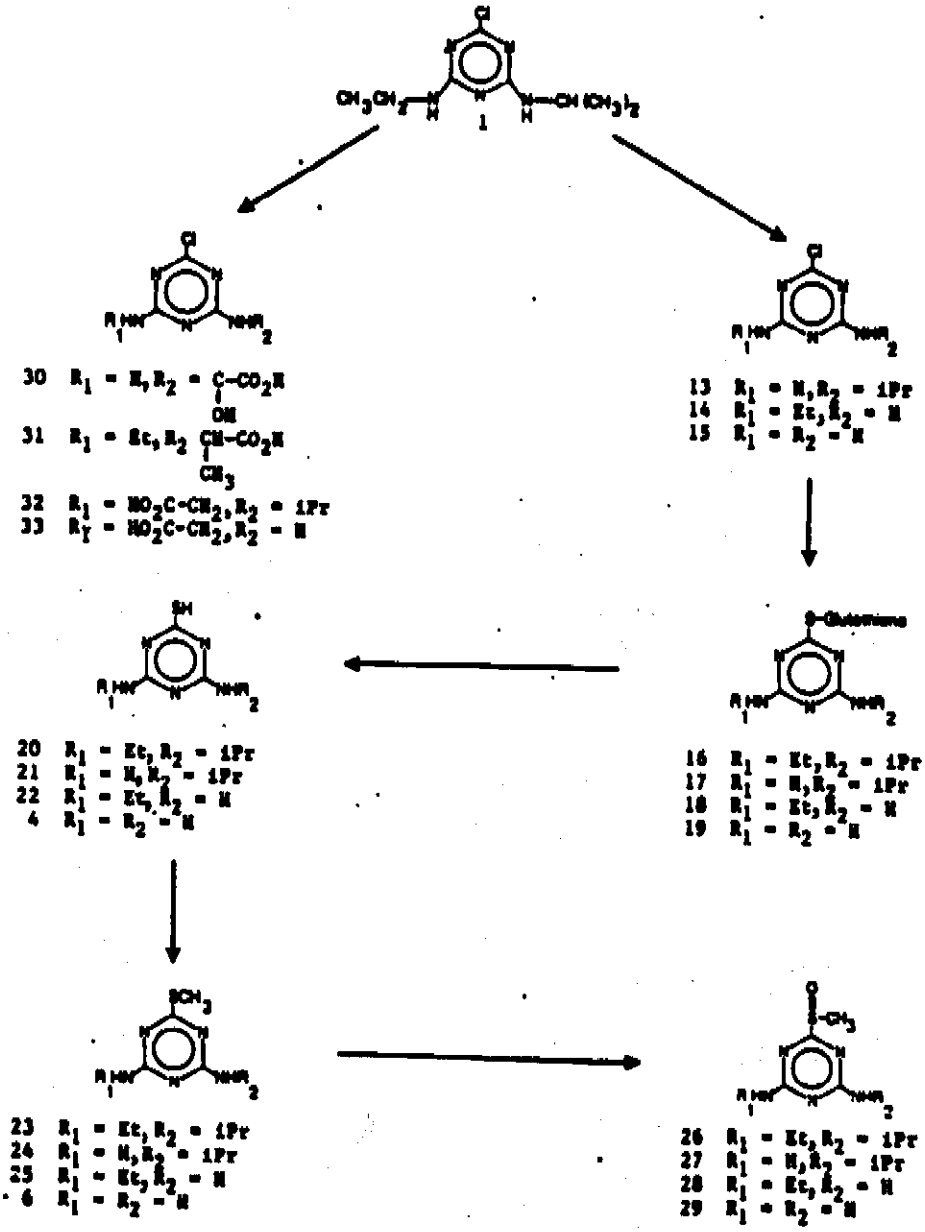
In vitro metabolism of atrazine. The author of this study offers the results of a published study on atrazine metabolism performed by Dauterman and Muecke (1974. Pesticide Biochemistry and Physiology 4:212-219) in an effort to account for the covalent binding in RBCs. To the reviewer's knowledge, the author of this study did not perform the work.

Radiolabeled atrazine was incubated with rat liver microsomes with or without the addition of the metabolic cofactors, glutathione and NADPH. Six metabolites were identified by chromatography against synthetic standards and are listed in figure 2. The published results corroborate the findings in the in vivo experiment conducted by the registrant that N-dealkylation is the major metabolic pathway. Also, the isopropyl moiety is hydrolyzed more easily than the ethyl substituent. Conjugation with glutathione was found to occur with most of the atrazine metabolites previously discussed when cytosolic cell fractions were included in the in vitro reactions.

Covalent binding in RBCs. The author argues that the glutathione-containing metabolites of atrazine are metabolized by a "carbon-sulfur lyase" that cleaves the glutathione residue and produces a thiol-containing atrazine metabolite. The author further posits that the action of the lyase results in the covalent binding of thiol-containing atrazine metabolites to hemoglobin in the red blood cell, a finding from the multiple dosing exposure studies (depicted in Table 7). However, the author has not discussed in this study whether lyase is present in red blood cells.

TAKEN FROM MRID NO. 404313-06

006718



160

FIGURE 2. PROPOSED METABOLIC PATHWAY FOR ATRAZINE IN THE RAT

V. **DISCUSSION:**

The summary data regarding the distribution, metabolism and the elimination of atrazine were provided in this report. To this end, three separate experiments were conducted with the use of three groups of rats. Radiolabeled atrazine (triazine ring, uniformly labeled) was used by the author to measure the disposition of atrazine and/or its metabolites in the rat. The first experiment was performed to assess the distribution and elimination of atrazine in male and female rats repeatedly exposed to daily doses of atrazine. The second experiment was performed to assess in further detail the distribution of atrazine in female rats, especially in the red blood cell. The third experiment was conducted to identify the urinary metabolites of atrazine formed by the female rat. The absorption of atrazine in male or female rats was not reported.

Absorption of atrazine in rats. No data was provided in this summary report regarding the absorption of atrazine in rats.

Distribution of Atrazine in rats. The distribution of atrazine in rats was found to be dose-dependent and independent of sex. Of the tissues studied, the red blood cells store the highest levels of atrazine, apparently through the covalent binding of a metabolite. In rats given a single dose of 100 mg/kg atrazine, in decreasing order, the levels found in the following tissues were: heart, spleen, lung, liver, kidney, brain, gonads, pituitary, muscle, uterus, bone, fat, and plasma. Under the exposure regimen used in this study, atrazine does not accumulate in the tissues of the rat, except perhaps for the red blood cell.

The pattern of tissue distribution of atrazine in rats repeatedly exposed differs from that of rats given a single exposure of atrazine. In rats given repeated daily oral doses of 1 mg/kg atrazine, in decreasing order, the levels found in the following tissues were: red blood cells, liver, spleen, kidney, lung, heart, pituitary, brain, gonads, muscle, bone, fat, and plasma. Under the exposure regimen used in this study, atrazine does not accumulate in the tissues of the rat, except perhaps for the red blood cell. The pattern of atrazine tissue distribution for atrazine in this study is similar to that found in rats repeated exposed to atrazine (see MRID Nos. 404313-04, 404313-05 and 404313-09 for more detail).

The distribution of atrazine was reported to follow first-order kinetics. Two major relationships are found: (1) the whole body half-life, or $t_{1/2}$, and the volume of distribution, or V_d, are independent of the dose of atrazine and (2) the plasma concentration of atrazine or its metabolites are directly

proportional to the dose of atrazine. Plasma concentrations of atrazine measured during and after atrazine exposure showed that the whole body half-life ($t_{1/2}$) of atrazine or its metabolites is 38.6 hours (1.61 days) in rats. These reported findings further indicate that atrazine does not accumulate under this exposure regimen in the rat.

As mentioned above, the highest level of atrazine was found in the RBC. The estimated half-life of 8.14 days in RBCs (as compared to the whole body half-life of 1.61 days) indicates that extensive binding of atrazine or its metabolites in RBCs was occurring. However, after cessation of multiple exposure, the concentration of atrazine or its metabolites in RBCs declined at all doses except for the highest dose, 100 mg/kg atrazine.

Excretion of atrazine in rats. About 95% of the atrazine administered orally is excreted within 7 days after cessation of exposure. The route of atrazine excretion was reported to be independent of the dose and sex of rat. About 75% of the atrazine is excreted through the urinary route whereas about 20% of the atrazine is eliminated in the feces. The elimination route for the remaining 5% was not reported. Also, the level of atrazine elimination by exhalation or through the skin (sweating) was not reported.

Metabolism of atrazine in rats. The data reported in this study indicates that dechlorination of the triazine ring and N-dealkylation are the major metabolic pathways for atrazine in rats. Oxidation of the alkyl substituents of atrazine appears to be a minor and secondary metabolic route.

The author argues that a "carbon-sulfur lyase," cleaves the glutathione residue from an atrazine metabolite to produce a thiol-containing atrazine metabolite. The author further posits that the action of the lyase results in the covalent binding of the thiol-containing atrazine metabolite to hemoglobin in the red blood cell, a finding from the multiple exposure studies (depicted in Table 7). However, the author has not provided evidence in this study whether lyase is present in red blood cells.

Summary. The whole body half-life of 1.61 days for atrazine is consistent with the observation that 95% of the administered dose is elimination within 7 days after exposure. The red cells store the highest concentration of atrazine in the rat, apparently through the covalent binding of a metabolite. Under the dose regimen employed in this study, atrazine does not accumulate in the rat.

Classification: Acceptable: This classification is based on the fact that the methodology requirements established in the Pesticide Assessment Guidelines, Subdivision F §85-1 have been satisfied only for reporting (1) the identity of urinary metabolites of atrazine in female rats as well as (2) the distribution and excretion of atrazine in male and female rats. However, all of the data requirements for metabolism studies set forth in Subdivision F §85-1 have not been reported, i.e., (a) the urinary and fecal metabolites of atrazine in male rats and (b) the fecal metabolites of atrazine in females must be identified to completely satisfy the §85-1 data reporting requirements for the metabolism of atrazine in the rat.

Reviewed by: Sanford W. Bigelow, Ph.D.
Section VI, Toxicology Branch (TS-769C)
Secondary reviewer: Judith W. Hauswirth, Ph.D.
Section VI, Toxicology Branch (TS-769C)

4/28/88

DATA EVALUATION REPORT

I. **SUMMARY:**

STUDY TYPE: Metabolism - rat (85-1) (and rabbit) CASWELL NO: 63

ACCESSION NUMBER: MRID NO.: 404313-13

TEST MATERIAL: Chemical Name Not Applicable

SYNONYMS: Atrazine Metabolites

STUDY NUMBER: Not Applicable

SPONSOR: CIBA-GEIGY Corp., Agricultural Division, P.O. Box 18300 Greensboro, NC 27419 Thomas Parshley, Regulatory Specialist (919) 292-7100 X7207

TESTING FACILITY: Max von Pettenkofer-Institut Berlin, Deutschland

TITLE OF REPORT: The Transformation of Triazine Herbicides in Animals. (Published Reference Information Supplemental to EPA Guideline 85-1.)

AUTHORS: C. Boehme and F. Baer

REPORT PUBLISHED IN: Cosmet. Toxicol. Volume 5, pp. 23-28 (1967).

CONCLUSIONS:

This study was submitted by the registrant in response to the reregistration standard for atrazine. The metabolism of atrazine, propazine, prometon and prometryn in rats and rabbits were reported in this study translated from a published article written in German. However, only the metabolism of atrazine was highlighted in this review. Numerous urinary metabolites of atrazine were isolated and identified in the rat and rabbit. The major pathway of atrazine metabolism is N-dealkylation and oxidation of the alkyl substituents appears to be a minor metabolic pathway. A classification for this study is not applicable because this is supplemental information.

II. MATERIALS:

A. Test Compound: Atrazine

Description: Not provided in this report.
Batch #: Not provided in this report.
Purity: Not provided in this report.

B. Test Animals:

Species: Rats (male), rabbits (male)
Strain: Rats - Albino, Rabbits - not provided in this report.
Age: Not provided in this report.
Weight: Rats - 200-300g, Rabbits - 2-3kg
Source: Rats - Max von Pettenkofer-Institut Berlin, Deutschland

III. STUDY DESIGN:

A. Feeding Mixture: The rats and rabbits were allowed free access to animal feed and tap water. Rats recieved a feed mixture that consisted of "700 g wheat yeast, 300 g whole milk powder, 350 ml water, 4 g dietary yeast and 10 g of a salt mixture." The rabbits received a diet of "boiled potatoes and beets."

B. Dosing Method: Atrazine and the other herbicides were given orally (via a stomach tube) as a single dose to the rats (50-200 mg/animal) and rabbits (600-1000 mg/animal) as an active ingredient. The vehicle was peanut oil.

C. Statistics:

There were no statistical procedures used in this study.

D. Quality Assurance:

A signed quality assurance statement was provided by a quality assurance inspector. According to the statement, because the "GLPs are not in effect for the study conducted in this volume, certification of compliance with Good Laboratory Practices is not applicable."

IV. METHODS AND RESULTS:

A. Observations: The frequency of clinical observations made on these rats was not provided in this summary report.

Toxicity/mortality (survival) results: There were no treatment-related deaths reported in this study.

B. Experimental Protocol: This experiment was conducted to identify the atrazine metabolites in rats and rabbits. The urinary metabolites were collected for 72 hours after exposure. The following methods were used to isolate and identify the metabolites of atrazine in the rats and rabbits:

(1) isolation by thin layer chromatography,

(2) characterization by ultraviolet or infrared spectra,

(3) confirmation of molecular structure by comparison to synthetic standards.

The organic chemistry methods that describe the syntheses of the standards are also reported.

The following metabolites of atrazine were identified in the rat and rabbit:

- 2-chloro-4,6-diamino-s-triazine,
- 2-chloro-4-amino-6-isopropylamino-s-triazine,
- 2-hydroxy-4-(2-carboxy)-ethylamino-6-amino-s-triazine, and
- 2-chloro-4-amino-6-(3-carboxy)-isopropylamino-s-triazine,

The identification of the four metabolites above indicates that N-dealkylation is the major metabolic pathway for atrazine in rats and rabbits. Because two atrazine metabolites that possess omega-carboxyl moieties were identified, oxidation of the terminal methyl moieties in the alkyl substituents appears to be a minor or secondary metabolic route in rats and rabbits.

[redacted]

V. **DISCUSSION:**

This study was submitted by the registrant in response to the reregistration standard for atrazine. The metabolism of atrazine, propazine, prometon and prometryn in rats and rabbits were reported in this study translated from a published article written in German. However, only the metabolism of atrazine was highlighted in this review. Numerous urinary metabolites of atrazine were isolated and identified in the rat and rabbit. The major pathway of atrazine metabolism is N-dealkylation and oxidation of the alkyl substituents appears to be a minor metabolic pathway. A classification for this study is not applicable because this is supplemental information.

Reviewed by: Sanford W. Bigelow, Ph.D.
Section VI, Toxicology Branch (TS-769C)
Secondary reviewer: Judith W. Hauswirth, Ph.D.
Section VI, Toxicology Branch (TS-769C)

006718
4/25/88
Judith W. Hauswirth
4/28/88

DATA EVALUATION REPORT

I. **SUMMARY:**

STUDY TYPE: Metabolism - rat (85-1) CASWELL NO: 63

ACCESSION NUMBER: MRID NO.: 404313-14

TEST MATERIAL: Atrazine

SYNONYMS: 2-Chloro-4-ethylamino-6-isopropylamino-s-triazine

STUDY NUMBER: Not Applicable

SPONSOR: CIBA-GEIGY Corp., Agricultural Division, P.O. Box 18300 Greensboro, NC 27419 Thomas Parshley, Regulatory Specialist (919) 292-7100 X7207

TESTING FACILITY: CIBA-GEIGY Corp., Agricultural Division Basle, Switzerland

TITLE OF REPORT: In Vitro Metabolism of Atrazine by Rat Liver. Published Reference Information Supplemental to EPA Guideline 85-1.

AUTHORS: W.C. Dauterman and W. Muecke

REPORT PUBLISHED IN: Pesticide Biochemistry and Physiology Volume 4, pp. 212-219 (1974).

CONCLUSIONS:

This report is a published study on atrazine metabolism performed by Dauterman and Muecke (1974. Pesticide Biochemistry and Physiology 4:212-219). This study was performed to study the in vitro metabolism of atrazine in the rat in an effort to ascertain which atrazine metabolite is responsible for covalent binding in RBCs.

This report of depicting these published results show that N-dealkylation is the major metabolic pathway for atrazine metabolism in the rat when investigated with the use of in vitro conditions. Also, the isopropyl moiety of atrazine is hydrolyzed more easily than the ethyl substituent. Conjugation with glutathione was found to occur with most of the atrazine metabolites when cytosolic cell fractions were included in the in vitro reactions.

[redacted]

168

Classification: This report was submitted by the registrant as part of a package in response to the reregistration standard for atrazine. This report constitutes supplemental information not required for the reregistration of atrazine.

II. MATERIALS:

A. Test Compound: Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine)

Description: Not provided in this report.
Batch #: Not provided in this report.
Purity: Not provided in this report for the nonradiolabeled compound.
Radiolabeling procedure:
All carbons in the triazine moiety of atrazine were replaced with carbon-14. The specific activity of the radiolabeled compound was 10.2 microCuries/mg. A variety of radiolabeled atrazine metabolites were used as well. The purity of the radiolabeled test compound was reported to be ≥ 99% ascertained by two different thin-layer chromatography systems.

B. Test Animals:

Species: Rats (male)
Strain: RAI
Age: Not provided in this study.
Weight: 150-170g
Source: CIBA-GEIGY Corp., Agricultural Division, Basle, Switzerland

III. STUDY DESIGN:

A. Animal Assignment:

Since this study was an in vitro study, no dosing of whole animals were performed. Therefore, animal assignment is not applicable in this study.

B. Statistics:

There were no statistical procedures used in this study.

C. Quality Assurance:

A signed quality assurance statement was provided by a quality assurance inspector. According to the statement, because the "GLPs are not in effect for the study conducted in this volume, certification of compliance with Good Laboratory Practices is not applicable."

[redacted]

IV. METHODS AND RESULTS:

A. The In Vitro Metabolism of Atrazine.

This published study on atrazine metabolism was performed by Dauterman and Muecke (1974. Pesticide Biochemistry and Physiology 4:212-219) in an effort to account for the covalent binding in RBCs.

The methods were reported as such. Radiolabeled atrazine was incubated with rat liver microsomes with or without the addition of the metabolic cofactors, glutathione and NADPH. Six metabolites were identified by chromatography against synthetic standards.

The published results show that N-dealkylation is the major metabolic pathway. Also, the isopropyl moiety of atrazine is hydrolyzed more easily than the ethyl substituent. Conjugation with glutathione was found to occur with most of the atrazine metabolites when cytosolic cell fractions were included in the in vitro reactions.

[redacted]

V. DISCUSSION

This report is a published study on atrazine metabolism performed by Dauterman and Muecke (1974. Pesticide Biochemistry and Physiology 4:212-219). This study was performed to study the in vitro metabolism of atrazine in the rat in an effort to ascertain which atrazine metabolite is responsible for covalent binding in RBCs.

This report of depicting these published results show that N-dealkylation is the major metabolic pathway for atrazine metabolism in the rat when investigated with the use of in vitro conditions. Also, the isopropyl moiety of atrazine is hydrolyzed more easily than the ethyl substituent. Conjugation with glutathione was found to occur with most of the atrazine metabolites when cytosolic cell fractions were included in the in vitro reactions.

Classification: This report was submitted by the registrant as part of a package in response to the reregistration standard for atrazine. This report constitutes supplemental information not required for the reregistration of atrazine.

006718

Reviewed by: Sanford W. Bigelow, Ph.D.
Section VI, Toxicology Branch (TS-769C)
Secondary reviewer: Judith W. Hauswirth, Ph.D.
Section VI, Toxicology Branch (TS-769C)

Judith W. Hauswirth 4/28/88

DATA EVALUATION REPORT

I. **SUMMARY:**

STUDY TYPE: Metabolism - rat (85-1) **CASWELL NO:** 63

ACCESSION NUMBER: **MRID NO.:** 404313-15

TEST MATERIAL: Chemical Name Not Applicable (Various s-Triazines)

SYNONYMS: Atrazine and Other s-Triazine Metabolites

STUDY NUMBER: Not Applicable

SPONSOR: CIBA-GEIGY Corp., Agricultural Division, P.O. Box 18300 Greensboro, NC 27419 Thomas Parshley, Regulatory Specialist (919) 292-7100 X7207

TESTING FACILITY: CIBA-GEIGY Corp., Agricultural Division Basle, Switzerland and Laboratorium fuer Biochemie I, Eidgenossische Technische Hochschule, 8092 Zurich, Switzerland.

TITLE OF REPORT: The Binding of s-Triazine Metabolites to Rodent Hemoglobins Appears Irrelevant to Other Species. (Published Reference Information Supplemental to EPA Guideline 85-1.)

AUTHORS: H. Hamboeck, R.W. Fischer, E.E. DiIorio, and K.H. Winterhalter.

REPORT PUBLISHED IN: Molecular Pharmacology Volume 20, pp. 579-584 (1981).

CONCLUSIONS:

The covalent binding of a variety of s-triazine metabolites to hemoglobin in a number of species was studied. The results regarding atrazine are highlighted in this review.

[redacted]

The relative level of covalent binding of atrazine to hemoglobin was lower when compared to most of the other s-triazine metabolites. The level of covalent binding, in decreasing order, of the atrazine metabolite to hemoglobin among the following animal species was: rat > guinea pig > dog > cow > mouse > human or sheep > pig.

Only the beta chain of hemoglobin was reported to covalently bind all of the s-triazine metabolites. The specific amino acid, the beta-125 cysteine (Cys-125), of hemoglobin was studied with simetryn sulfoxide only, therefore, the only results stemming from this amino acid analysis deal with simetryn sulfoxide and not atrazine. The authors report that Cys-125 resides on the outer surface of hemoglobin in the rat and guinea pig, however, the stereochemistry of human hemoglobin was not discussed.

Classification: This report was submitted by the registrant as part of a package in response to the reregistration standard for atrazine. This report constitutes supplemental information not required for the reregistration of atrazine.

II. MATERIALS:

A. Test Compound:

Description: Not provided in this study.
Batch #: Not provided in this study.
Purity: Not provided in this study.

B. Test Animals:

Species: Rat, mouse, guinea pig, human, sheep, cow, pig, and chicken.
Strain: Not reported in this study.
Age: Not reported in this study.
Weight: Not reported in this study.
Source: Sheep, cows, and pigs were from a slaughterhouse.

III. STUDY DESIGN:

A. Animal Assignment:

Since this study was an in vitro study, no dosing of whole animals were performed. Therefore, animal assignment is not applicable in this study.

B. Statistics:

There were no statistical procedures used in this study.

C. Quality Assurance:

A signed quality assurance statement was provided by a quality assurance inspector. According to the statement, because the "GLPs are not in effect for the study conducted in this volume, certification of compliance with Good Laboratory Practices is not applicable."

IV. METHODS AND RESULTS:

The covalent binding of a variety of s-triazine metabolites to hemoglobin in a number of species was studied. The following s-triazine herbicides were studied: simetryn, simetryn sulfoxide, desaethylsimetryn, ametryn, ametryn sulfoxide, dimethametryn, dipropetryn, simazine and atrazine. The results regarding atrazine are highlighted in this review.

Blood was obtained from the following animal species: rat, mouse, guinea pig, human, sheep, cow, pig, and chicken. The red blood cells from each species were separated and lysed to yield hemoglobin. The rat liver microsomal oxidation products (metabolites) of the above s-triazine compounds were allowed to react with the hemoglobin from each species. After the reaction had taken place, the (1) overall level of covalent binding of s-triazine metabolite to hemoglobin was measured and (2) the specific amino acid residue in hemoglobin which covalently bound the s-triazine metabolite was identified.

The relative level of covalent binding of atrazine to hemoglobin was lower when compared to most of the other s-triazine metabolites. The level of atrazine metabolite covalent binding to hemoglobin, in decreasing order, among the following species was: rat > guinea pig > dog > cow > mouse > human or sheep > pig.

Only the beta chain of hemoglobin was reported to covalently bind all of the s-triazine metabolites. The specific amino acid, the beta-125 cysteine (Cys-125), of hemoglobin was studied with simetryn sulfoxide only, therefore, the only results stemming from this amino acid analysis deal with simetryn sulfoxide and not atrazine. The authors report that Cys-125 resides on the outer surface of hemoglobin in the rat and guinea pig, however, the stereochemistry of human hemoglobin was not discussed.

V. **DISCUSSION**:

The covalent binding of a variety of s-triazine metabolites to hemoglobin in a number of species was studied. The results regarding atrazine are highlighted in this review.

The relative level of covalent binding of atrazine to hemoglobin was lower when compared to most of the other s-triazine metabolites. The level of covalent binding, in decreasing order, of the atrazine metabolite to hemoglobin among the following animal species was: rat > guinea pig > dog > cow > mouse > human or sheep > pig.

Only the beta chain of hemoglobin was reported to covalently bind all of the s-triazine metabolites. The specific amino acid, the beta-125 cysteine (Cys-125), of hemoglobin was studied with simetryn sulfoxide only, therefore, the only results stemming from this amino acid analysis deal with simetryn sulfoxide and not atrazine. The authors report that Cys-125 resides on the outer surface of hemoglobin in the rat and guinea pig, however, the stereochemistry of human hemoglobin was not discussed.

Classification: This report was submitted by the registrant as part of a package in response to the reregistration standard for atrazine. This report constitutes supplemental information not required for the reregistration of atrazine.

006718

Reviewed by: Sanford W. Bigelow, Ph.D. [signature] 4/25/88
Section VI, Toxicology Branch (TS-769C)
Secondary reviewer: Judith W. Hauswirth, Ph.D. Judith W. Hauswirth 4/25/88
Section VI, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

I. **SUMMARY:**

STUDY TYPE: Metabolism - rat (85-1) **CASWELL NO:** 63

ACCESSION NUMBER: **MRID NO.:** 404313-12

TEST MATERIAL: Chemical Name Not Applicable

SYNONYMS: Propazine and Prometone Metabolites

STUDY NUMBER: Not Applicable

SPONSOR: CIBA-GEIGY Corp., Agricultural Division, P.O. Box 18300 Greensboro, NC 27419 Thomas Parshley, Regulatory Specialist (919) 292-7100 X7207

TESTING FACILITY: USDA, ARS, Metabolism and Radiation Research Lab, Fargo, ND 58102.

TITLE OF REPORT: Metabolism of 2-Chloro-4.6-bis(isopropylamino)-s-triazine (Propazine) and 2-Methyl-4,6-bis(isopropylamino)-s-triazine (Prometone) in the Rat. Balance Study and Urinary Metabolite Separation. (Published Reference Information Supplemental to EPA Guideline 85-1.)

AUTHORS: J.E. Bakke, J.D. Robbins, and N.J. Feil

REPORT PUBLISHED IN: Journal of Agricultural and Food Chemistry Volume 15, pp. 628-631 (1967).

CONCLUSIONS:

This study was submitted by the registrant in response to the reregistration standard for atrazine. However, this study does not report the metabolism of atrazine. Because this study involves the metabolism of propazine and prometone and not atrazine, this study is of little use in evaluating the metabolism of atrazine in the rat. A classification for this study is not applicable because this is supplemental information not required for the reregistration of atrazine.

II. MATERIALS:

A. Test Compounds: Propazine and prometone

Description: Not provided in this study.
Batch #: Not provided in this study.
Purity: Not provided in this report for the nonradiolabeled compound.
Radiolabeling procedure:
The carbons in the isopropyl moiety of atrazine were replaced with carbon-14. The specific activity of radiolabeled propazine was 9.33 microCurie/mg. The specific activity of radiolabeled prometone was 9.33 microCurie/mg. The purity of either radiolabeled test compound was not reported.

B. Test Animals:

Species: Rat (males)
Strain: Sprague-Dawley
Age: Not provided in this study.
Weight: Not provided in this study.
Source: Delmar Scientific Laboratories, Chicago, IL

III. STUDY DESIGN:

A. Animal Assignment: Animals were individually assigned to a metabolism cage once the test compound was administered.

B. Feeding Mixture: The rats and rabbits were allowed free access to animal feed (brand name not specified) and tap water.

C. Dosing Method: Propazine was given orally (via a stomach tube) as a single dose (1-5 microCurie/rat) to the rats (41-56 mg/kg) as an active ingredient. Prometone was given orally (via a stomach tube) as a single dose (0.75 microCurie/rat) to the rats (20.8-25.3 mg/kg) as an active ingredient. Corn oil was used as a vehicle. The stability of the compounds were not affected by heating in corn oil as corroborated by ultraviolet and infrared spectroscopy, gas chromatography and thin layer chromatography.

D. Statistics:

There were no statistical procedures used in this study.

E. Quality Assurance:

A signed quality assurance statement was provided by a quality assurance inspector. According to the statement, because the "GLPs are not in effect for the study conducted in this volume, certification of compliance with Good Laboratory Practices is not applicable."

IV. METHODS AND RESULTS:

A. Observations: The frequency of clinical observations made on these rats was not provided in this summary report.

Toxicity/mortality (survival) results: There were no treatment-related deaths reported in this study.

B. Experimental Protocol: This experiment was conducted to isolate the propazine and prometone metabolites produced in rats. The urinary and fecal metabolites were collected for 72 hours after exposure. The expired air of the rats was monitored for radioactivity. Two methods were used to isolate the metabolites of propazine and prometone in rats, ion exchange column chromatography and amino acid analysis.

The excretion of prometone and propazine was most rapid within 24 hours after administration and diminished to trace levels at 72 hours. All of the radioactive prometone was recovered whereas 94.3% of the propazine was recovered. No radioactivity was found in the expired air of the rats. All of the prometone was excreted in the urine (91.1% and feces (9.1%) within 72 hours after exposure. Propazine was excreted slower than prometone, at 72 hours, 65.8% was found in the urine and 23.% was found in the feces. At 12 days after propazine exposure, urine had 72.5%, feces had 15.6%, hide and hair had 3.35%, carcass had 2.22% and the viscera had 0.1%. Propazine is eliminated more slowly than prometone.

Ion exchange chromatography techniques were used to detect 11 metabolites for prometone and 18 metabolites for propazine. None of the structures of the prometone and propazine metabolites were identified.

The findings in this study indicate that N-dealkylation may be the major metabolic pathway for propazine and prometone in rats.

V. **DISCUSSION:**

This study was submitted by the registrant in response to the reregistration standard for atrazine. However, this study does not report the metabolism of atrazine. Because this study involves the metabolism of propazine and prometone and not atrazine, this study is of little use in evaluating the metabolism of atrazine in the rat. A classification for this study is not applicable because this is supplemental information not required for the reregistration of atrazine.

006718

Reviewed by: Sanford W. Bigelow, Ph.D. 4/27/88
Section VI, Toxicology Branch (TS-769C)
Secondary reviewer: Judith W. Hauswirth, Ph.D. Judith W. Hauswirth 4/28/88
Section VI, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

I. SUMMARY:

STUDY TYPE: Metabolism - rat (85-1) CASWELL NO: 63

ACCESSION NUMBER: MRID NO.: 404313-16

TEST MATERIAL: Chemical Name Not Applicable

SYNONYMS: 2-Chloro-N-isopropylacetanilide (Propachlor)

STUDY NUMBER: Not Applicable

SPONSOR: CIBA-GEIGY Corp., Agricultural Division, P.O. Box 18300 Greensboro, NC 27419 Thomas Parshley, Regulatory Specialist (919) 292-7100 X7207

TESTING FACILITY: USDA, ARS, Metabolism and Radiation Research Lab, Fargo, ND 58105

TITLE OF REPORT: Metabolism of Mercapturic Acid-Pathway Metabolites of 2-Chloro-N-isopropylacetanilide (Propachlor) by Gastrointestinal Bacteria. (Published Reference Information Supplemental to EPA Guideline 85-1.)

AUTHORS: G.L Larsen and J.E. Bakke

REPORT PUBLISHED IN: Xenobiotica Volume 13, Issue No. 2, pp. 115-126 (1983).

CONCLUSIONS:

This report submitted by the registrant in response to the reregistration standard for atrazine. This report is a published study that shows the gastrointestinal bacteria of the pig possesses lyase enzyme. Because this study involves the metabolism of 2-chloro-N-isopropyl-acetanilide (propachlor) and not atrazine, this study is of little use in evaluating the metabolism of atrazine or even its thiol-containing metabolites in the rat. This report constitutes supplemental information not required for the reregistration of atrazine.

PC1/reports/atrametb.014

006718

Reviewed by: Sanford W. Bigelow, Ph.D.
Section VI, Toxicology Branch (TS-769C)
Secondary reviewer: Judith W. Hauswirth, Ph.D.
Section VI, Toxicology Branch (TS-769C)

4/23/88

DATA EVALUATION REPORT

I. **SUMMARY:**

STUDY TYPE: Metabolism - rat (85-1) **CASWELL NO:** 63

ACCESSION NUMBER: **MRID NO.:** 404313-17

TEST MATERIAL: Chemical Name Not Applicable

SYNONYMS:

STUDY NUMBER: Not Applicable

SPONSOR: CIBA-GEIGY Corp., Agricultural Division, P.O. Box 18300 Greensboro, NC 27419 Thomas Parshley, Regulatory Specialist (919) 292-7100 X7207

TESTING FACILITY: Univ. of Chicago, Dept. of Pharmacology, Chicago, IL

TITLE OF REPORT: Metabolism Cage for Rats. (Published Reference Information Supplemental to EPA Guideline 85-1.)

AUTHOR: L.J. Roth

REPORT PUBLISHED IN: Nucleonics p. 104 (1956).

CONCLUSIONS:

The report submitted by the registrant is a published article that describes the design of a metabolism cage. This cage allows the investigator to collect the urine, feces and the expired air of the animal. This metabolism cage may have been used by the registrant (or its subcontractors) to study the distrbution, metabolism, and excretion of atrazine, but this is a presumption.

PC1/reports/atrametb.016

END